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REPORTS

OF THE

COMMISSION

APPOINTED BY

THE ADMIRALTY, THE WAR OFFICE, AND
THE CIVIL GOVERNMENT OF MALTA,

FOR THE INVESTIGATION OF

MEDITERRANEAN FEVER,

UNDER THE SUPERVISION OF AN

ADVISORY COMMITTEE

OF

THE ROYAL SOCIETY.

PART VI.

LONDON:
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Early Science
RC 106
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CONTENTS.

PAGE

I. Report upon the Bacteriological and Experimental Investigations during the Summer of 1906. By J. W. H. Eyre, M.D., F.R.S. Edin., Bacteriologist to Guy's Hospital and Lecturer on Bacteriology in the Guy's Medical School; Major J. G. McNaught, M.D., R.A.M.C.; Captain J. C. Kennedy, M.B., R.A.M.C.; and T. Zammit, M.D., Government Analyst, Malta, and Professor of Chemistry, Malta University 3

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I. REPORT UPON THE BACTERIOLOGICAL AND EXPERIMENTAL INVESTIGATIONS DURING THE SUMMER OF 1906.

By J. W. H. Eyre, M.D., F.R.S. Edin., Bacteriologist to Guy's Hospital and Lecturer on Bacteriology in the Guy's Medical School; Major J. G. McNaught, M.D., R.A.M.C.; Captain J. C. Kennedy, M.B., R.A.M.C.; and T. Zammit, M.D., Government Analyst, Malta, and Professor of Chemistry, Malta University.

SYNOPSIS OF CONTENTS.

Page
Preliminary Notes on Methods 4

I.—The Goat as a Factor in the Dissemination of Malta Fever.

1. The distribution of milch goats naturally infected with *Micrococcus melitensis* 7
2. The agglutination test as applied to the milch goat 14
3. The numerical relationship of *M. melitensis* to the milk of infected goats 19
4. The presence of *M. melitensis* in milk products 25
5. The susceptibility of the goat to, and path of infection by, *M. melitensis* 28
6. The localisation of *M. melitensis* in the infected goat 36
7. The transmission of *M. melitensis* antibodies to the descendants of infected goats 41
8. The treatment of infected milch goats with *M. melitensis* vaccine 42

II.—*M. melitensis* Infection by Experimental Feeding with Infected Goats' Milk.

1. General considerations 44
2. Feeding experiments with naturally infected milk artificially reinforced 48
3. Feeding experiments with naturally infected milk alone 57
4. Clinical features of food infection (*M. melitensis*) in the monkey.. 65
5. The action of hydrochloric acid and of artificial gastric juice on *M. melitensis* 68
III.—*M. melitensis* infection following the ingestion of infective milk in man 70

Résumé of the outbreak of Mediterranean Fever on board the ss. "Joshua Nicholson."

1. History of the goats 70
2. Incidence of Mediterranean Fever among those who partook of the milk 71
3. The results 72

PAGE
IV.—The *Rôle* of the Mosquito and other Blood-sucking Insects in the Dissemination of *M. melitensis*.
1. Species of mosquitoes occurring in Malta 73
2. Supply of mosquitoes, and notes on their habits in captivity 77
3. Duration of life of *M. melitensis* in the body of the mosquito ... 80
4. Experiments with *Acartomyia Zammitii* 82
Duration of life of *M. melitensis* in Acartomyia 83
Virulence of *M. melitensis* after passage through Acartomyia 85
Acartomyia as the infective agent...................................... 85
5. Experiments with *Stegomyia fasciata* 91
Duration of life of *M. melitensis* in Stegomyia 91
Stegomyia as the infective agent 92
6. Experiments with other species of mosquitoes 94
7. Biting flies, *Stomoxys calcitrans* 96
Duration of life of *M. melitensis* in Stomoxys........................ 96
Stomoxys as the infective agent.. 97
8. Fleas and bugs 99
V.—Naturally acquired infection (*M. melitensis*) in various animals 101
VI.—*M. melitensis* infection by means of Personal Contact 107
VII.—Prophylactic Vaccination with *M. melitensis* Vaccine.
1. The subjects selected for vaccination and the results 115
2. The vaccine 116
3. The inoculations 119
4. Clinical phenomena.. 120
5. Tabular details... 121
VIII.—Clinical Observations on Malta Fever.. 123
IX.—Conclusions and Recommendations 130

PRELIMINARY NOTES ON METHODS.

A brief summary of the technique employed for the isolation and identification of *M. melitensis*, the performance of the agglutination test, and the selection of experimental animals, is inserted here in order to avoid unnecessary repetition in the body of the Report.

Isolation of M. melitensis.

1. *Medium.*—Nutrient peptone broth, prepared from Brand's Essence of Beef and standardised to +10 (Eyre's scale), was frequently used for the preliminary enrichment of blood, etc., but the medium generally employed for the isolation of *M. melitensis*, and except where otherwise stated, was a nutrose-litmus-agar prepared on a basis of peptone broth, containing nutrose to the extent of about 1 per cent., tinted with litmus solution to an arbitrarily chosen colour standard, and standardised so far as concerns reaction to +10 (Eyre's scale).

This was the medium employed by the members of the Commission during the years 1904 and 1905; and although by no means the optimum medium, it was decided to continue its use throughout 1906 in order to render the results absolutely comparable with those of former workers.

2. *Method of Culture.*—This medium was used in the form of surface "plates"—the liquefied agar being poured into Petri dishes, allowed to "set," and, as a preliminary to inoculation, incubated for 24 hours at 37° C., so that any accidentally contaminated plates might be detected and discarded. In preparing plates, the infected material was deposited near the centre of the surface of the nutrose-agar and then distributed over the entire surface of the medium by the aid of a sterile L-shaped glass spreader. In the case of grossly-contaminated material, several plates were prepared *in series*—that is to say, after the first plate had been inseminated the same spreader was employed to inoculate a second, then a third, and even a fourth and fifth with the traces of infective material still adhering to it—a method which yields dilutions comparable to those prepared by measuring with loopfuls when the liquefied medium in tubes is inoculated and then used for pouring plates.

Incubation was invariably carried out aerobically at 37° C., and in those cases where colonies of *M. melitensis* had not developed by the second or third day, all plates were studied for at least seven days—except where the plates were so grossly contaminated as to be unworkable.

Identification of M. melitensis.

Suspicious colonies of cocci developing on these plates were first tested with serum derived either from patients suffering from Malta Fever or from experimental animals, in dilution of, usually, 1 in 100. Such as responded to this test and yielded a positive agglutination reaction were sub-cultivated in tubes of ordinary agar (+10), and the subsequent growths, before being recorded as due to *M. melitensis*, were required to conform to the following criteria:—

1. Size, shape, and Brownian activity	Corresponding to those exhibited by authentic type cultures.
2. Readiness and homogeneity of emulsion	

3. Non-retention of stain when treated by Gram's method.

4. Production of alkaline reaction without change of consistency in litmus milk.

5. Complete clumping when tested with specific agglutinating serum in high dilution: the dilution varied with the maximum titre of the serum employed, but was usually 1 in 1000.

In many cases the cocci under examination were further tested as to their ability to produce *M. melitensis septicæmia* in the guinea-pig when inoculated intracerebrally.

Performance of Agglutination Reactions.

The culture employed was invariably an early sub-culture grown on ordinary +10 agar in "sloped" tubes and usually for 24 or 48 hours at 37° C., from a strain of *M. melitensis* that had recently been isolated from the animal body. Occasionally, but very rarely, it was found necessary to use three-day-old cultures, but the use of such old cultures was discouraged as much as possible.

The growth was emulsified in sterile 0·1 per cent. salt solution or in sterile distilled water, in the proportion approximately of 0·5 milligramme of bacterial growth to 1 c.c. of fluid.

The serum to be tested was diluted with normal saline solution and deposited in a series of small test-tubes. The dilutions prepared started with 1 in 5 (20 per cent.), and 1 in 10 (10 per cent.), and proceeded upwards in tenths of the first named—*e.g.*, 1 in 50 (2 per cent.), 1 in 500 (0·2 per cent.), and so on, so that when equal quantities of emulsion and diluted serum were mixed, dilutions of 1 in 10, 1 in 20, 1 in 100, 1 in 1000, etc., were available for examination, other secondary dilutions, 1 in 50, 1 in 200, etc., being prepared and examined as occasion required.

The examination was carried out both microscopically in the hanging drop, and macroscopically in sealed Pasteur pipettes—each method of observation being carefully controlled by a blank preparation consisting of equal quantities of normal salt solution and emulsion of *M. melitensis.*

In the microscopical examination, the period of observation was limited to 30 minutes, and the result recorded as positive (+) when *all* the cocci were agglutinated into large clumps and none remained free in the fluid; as incomplete (±) when the clumps were small, and many cocci, singly and in pairs, were scattered about the field; and as negative (–) when clumps were very small, comprising only a dozen or so individual cocci, or were completely absent.

In the macroscopical examination, the period of observation was extended, if necessary, to 24 hours, and a positive result recorded only when the supernatant fluid was quite clear and all the cocci had sedimented into a compact mass at the point of the pipette; a loose mass of cocci below and slightly turbid fluid above was returned as an incomplete reaction, and where the contents of the pipette closely resembled those of the control a negative reaction was recorded. In only a very few of the many thousands of preparations put up during the course of the summer did the macroscopical and microscopical results fail to control and confirm each other.

Selection of Animals for Experimental Work.

Healthy animals, such as goats, kids, and monkeys, after purchase, were placed in the Lazzaretto as soon as they reached the Commission. Here they were tethered—out of reach of each other in the various stables and rooms which had been rendered fly- and mosquito-proof by covering the windows with fine gauze and supplying the doorways with mosquito-netting curtains—away from either infected animals or healthy animals already stabled there. The rectal temperature was taken night and morning; the blood repeatedly examined for the presence of specific agglutinins, and, in the case of milch goats, the milk examined for the presence of the *M. melitensis*. Only when the temperature showed an absence of marked excursions from the accepted normal, and no evidence of infection with *M. melitensis* could be detected, were the animals regarded as "healthy and normal," and as such used for the experimental work of the Commission.

I.—THE GOAT AS A FACTOR IN THE DISSEMINATION OF MEDITERRANEAN FEVER.

1. *The Distribution of Milch Goats Naturally Infected with* M. melitensis.

The work of the previous year in collecting information respecting the distribution of infected goats by examining herds from various parts of the island was continued as opportunity afforded in 1906. In the first place, the milk supply of various military centres and the village of Lia were investigated with the following results:—

Table I.—The Proportion of Healthy and Infected Milch Goats in various Herds.

Herd supplying.	No. of goats in herd.	Milk reaction in	*M. melitensis* in milk of
1st Rifle Brigade	46	7	1
West Kent Regiment	40	10	3
(Re-examination of reacting goats)...		(3)	(0)
Citta Vecchia Sanatorium*	38	12	3
Forrest Hospital†	8	0	0
Imtarfa Hospital	60	2	0
Valletta Hospital‡........................	18	6	1
Lia	85	8	0
Total	295	45	8
Percentage	...	15·2	2·7
* The corresponding figures for 1905 were	15	11	5
† ,, ,, ,,	15	5	1
‡ ,, ,, ,,	13	4	1

The small number of goats (18 animals) examined in connection with Valletta Hospital comprised only about one-quarter of the goats actually supplying milk to the hospital, but the passive resistance of the goatherds in relation to the collection of samples, which was in no way overcome by the loud protestations of the contractor, resulted in the absence of the remainder of the goats whenever an attempt was made to collect specimens of milk. Within a few days of the examination of this small portion of the herd a general strike of the goatherds supplying milk to Valletta was instituted and maintained from May 14th until June 1st, 1906. The strike lasted, in fact, until all the military hospitals and the majority of other large consumers had been compelled to replace their supplies of goats' milk by various brands of condensed or other tinned milk. Having thus forfeited their contracts and being no longer in the position of supplying milk to the hospitals and regiments, all hold over the goatherds was lost, and it was subsequently found to be impossible to complete these examinations in which a portion only of a herd had been investigated.

A comparison with the results obtained in 1905* shows that the average number of infected animals, per herd, was much smaller than that noted when large numbers distributed over more extensive areas were dealt with, and forcibly illustrates the fallacy which would attend generalisations from the results obtained above.

At the commencement of the season's work when comparing the incidence of Malta Fever upon the civil population of the island in the various local centres, the severity of this incidence upon certain villages, and the apparent absence of the disease from others closely adjacent, was particularly noticeable, and it was decided to carefully investigate the milk supply of Rabato, a suburb of Citta Vecchia, and to remove from time to time such goats as were found to be discharging *M. melitensis* in their milk from the herds examined, and study the effects of the removal of so much infective material from a restricted area upon the incidence of the fever.

This plan was at once put into execution, and a preliminary examination of the Rabato herds gave the following results :—

Table II.—Infected Goats at Rabato.

No. of herds examined.	No. of goats examined.	No. of milks reacting.	No. of milks containing *M. melitensis*.
49	342	52	16
	Percentage......	15	4·6

---

* *Vide* Zammit, these Reports, IV, 96 *et seq.*; Horrocks and Kennedy, IV, p. 37 *et seq.*

At first all went well. Samples were readily supplied, and the 16 infected goats were handed over by their owners for observation and treatment at the Lazzaretto, where each was supplied with a collar bearing a metal disc on which was stamped a serial number, to aid in the ready identification of the individual goats. The serial numbers commenced at 101 in order to avoid any confusion with experimental and other goats remaining at the Lazzaretto from previous years. (These animals were subsequently purchased at valuation and were utilised for experimental observations throughout the summer.) Soon, however, the influence of the goatherds' strike in Valletta spread to the provincial districts, and the goatherds of those villages in which operations were being carried on, refused in their turn to allow milk samples to be taken.

Unfortunately, the local ordinances and statutes regulating the supply of food stuffs take little active cognisance of the goat as a source of milk, and proved totally inadequate—at any rate from a diplomatic standpoint—to enforce compliance with the requests of members of the Working Party even when supported by sanitary inspectors, and the observations had perforce to be abandoned. The details of the examinations that were carried out are, however, inserted in Table III, because from them, incomplete though they are, emerge one or two suggestive points of more than passing interest.

A subsidiary matter was to have been the investigation of those milch goats which, by the presence of specific agglutinins in their body fluids, gave evidence of infection, recent or remote, by *M. melitensis*, but whose milk did not contain the micro-organism. It was hoped that by a careful study of these animals some criteria might be arrived at by which it would be possible to determine whether the infection was so remote that the milk would remain innocuous, or so recent that the appearance of *M. melitensis* in the milk would be an ever-present danger.

This investigation likewise had to be temporarily abandoned with the larger experiment.

During the course of the investigations, perhaps the most striking observation recorded was the healthy appearance of the majority of the infected goats. The animals were sleek and plump, with smooth, healthy-looking coats; they took their food well, were as active as their uninfected fellows, and yielded as large a quantity of milk and of apparently as good a quality. Palpation failed to reveal any enlargement of lymphatic glands or of alteration of the mammary gland. In many instances the infected milch goats were the best looking and the best milkers in the herd, and in a few instances only it was noted that an infected animal suffered from a short barking cough at infrequent intervals.

Perhaps the most noteworthy feature in the following table is the

Table III.—Examination of Herds at Rabato.

No. of herd.	Name of owner.	No. of goats in herd.	No. of milks reacting.	No. of milks containing *M. melitensis.*	Cases of Malta Fever on the premises during the current 12 months.
1	F. Vassallo	2	0	—	—
2	M. Borg	2	0	—	—
3	P. Cutajar	15	0	—	—
4	G. Mifsud	3	0	—	—
5	G. Agius	24	9	4	1
6	A. Micallef	4	0	—	—
7	G. Vassallo	5	0	—	—
8	P. D'Anatas.........	3	0	—	—
9	G. Ciantar	4	0	—	—
10	G. Attard............	2	0	—	—
11	S. Micallef	7	2	1	1
12	L. Micallef	4	0	—	—
13	G. Galea	6	2	1	1
14	G. Azzopardi	5	3	2	2
15	L. Sant...............	11	4	—	1
16	G. Attard............	8	2	—	1
17	F. Micallef	5	0	—	—
18	P. Sant...............	22	6	—	1
19	G. Grech	9	0	—	—
20	G. Inguanez.........	5	0	—	—
21	G. Micallef	8	0	—	—
22	P. Micallef	2	0	—	—
23	F. Galea	14	1	—	—
24	G. M Azzopardi...	7	1	—	—
25	S. Pace	12	0	—	—
26	C. Formosa	13	0	—	—
27	G. Micallef	4	1	—	1
28	V. Azzopardi	7	1	—	1
29	G. Vassallo	2	0	—	—
30	P. Borg...............	4	1	—	—
31	C. Borg...............	2	0	—	—
32	G. Micallef	1	0	—	—
33	G. Azzopardi	12	0	—	—
34	A. Dimech	5	0	—	—
35	F. Micallef	4	0	—	—
36	P. Borg...............	1	0	—	—
37	A. Dimech	1	0	—	—
38	V. Borg	17	10	7	2
39	C. Borg...............	29	2	—	—
40	Carmela Mifsud ...	9	4	1	*
41	G. Zahra	14	4	1	5
42	F. Vassallo	5	0	—	—
43	F. Portelli	2	0	—	—
44	S. Camilleri.........	2	0	—	—
45	C. Calleja............	3	0	—	—
46	V. Attard............	5	0	—	—
47	P. Formosa	9	1	—	1
48	C. Mifsud............	4	0	—	—
49	S. Dingli	2	0	—	—

* In close association with next-door neighbour and relative (G. Zahra), owner of Herd No. 41, in whose house five cases have occurred.

occurrence of one or more cases of Malta Fever during the present twelvemonth in the household of every owner of a milch goat, which, by reason of the isolation of the *M. melitensis* from its milk, was conclusively proved to be infected, with one exception, viz., in the case of Herd No. 40, whose owner occupied the adjoining house to the owner of Herd No. 41. Here the two families and the two herds were intimately acquainted and in close daily contact, and in the house of the owner of Herd No. 41 no less than five cases of Malta Fever have occurred.

This association of cases of Malta Fever in man with infected milch goats suggests in a striking manner the highly infective character of the milk cultivation—usually practically pure—of *M. melitensis* yielded by many of these milch goats, for the Maltese goatherd keeps his goats for profit and rarely uses goat's milk as a food for himself or his family; and we are, personally, convinced from our enquiries and observations that, in the vast majority of cases occurring in goatherds and their families, infection is contracted during the handling of this food by the direct inoculation of infected milk into cuts, scratches and abrasions on the face, hands, forearms, feet and legs of the individual.

Cases were also present in some of the households where the herds contained milch goats, which, although yielding a positive agglutination reaction at the time of examination, were not passing the micro-organism in the milk. This, however, is no matter for surprise, and in no way militates against the views expressed above, for, as it will be mentioned later, it is not uncommon for a milch goat to yield milk one day containing more than 30,000 *M. melitensis* per cubic centimetre, and a few days later, milk apparently quite free from the microbe.

It has already been stated that many of these herds at Rabato were examined more than once, some a second time three weeks after the preliminary examination, and a few on a third occasion a fortnight later, before the milk vendors' strike became general. These re-examinations and their results are set out in Tables IV and V.

Inspection of these tables shows also the variation in size that individual herds undergo during comparatively short periods, in the present instances quite apart from the diminution in numbers resulting from the removal of those goats whose milk yielded *M. melitensis* from their respective herds to the experimental stables at the Lazzaretto.

The figures quoted were obtained by actual observation and by comparison with the Registration Books kept at the local police headquarters; for a system is in vogue under which every goat owner reports, personally or by proxy, the number of goats in his possession every Saturday to the police. The number thus reported includes kids, male goats, and pregnant females not yielding milk as well as milch goats in full milk, the object of registration being to assist the Sanitary Service in the event of the occurrence of an epidemic of foot-and-mouth disease, by calling attention to any diminution in the number of

Table IV.—Re-Examination of certain Herds at Rabato.

No. of herd.	No. of examination.	No. of goats in herd.	No. of milks reacting.	No. of milks containing *M. melitensis.*
3	First	15	0	0
	Second	10	0	0
4	First	3	0	0
	Second	3	0	0
5	First	24	9	4
	Second	15	5	1
6	First	4	0	0
	Second	3	0	0
15	First	11	4	0
	Second	11	0	0
16	First	7	1	0
	Second	8	1	1
17	First	5	0	0
	Second	5	0	0
19	First	9	0	0
	Second	6	0	0
20	First	5	0	0
	Second	2	0	0
21	First	8	2	0
	Second	7	1	0
22	First	2	0	0
	Second	2	0	0
23	First	14	1	0
	Second	8	0	0
24	First	7	1	0
	Second	5	0	0
25	First	12	0	0
	Second	12	0	0
27	First	4	1	0
	Second	4	0	0
28	First	7	0	0
	Second	7	1	0
29	First	2	0	0
	Second	2	0	0
30	First	4	1	0
	Second	5	0	0
33	First	11	0	0
	Second	10	0	0
40	First	9	4	1
	Second	6	1	0
41	First	14	4	1
	Second	10	1	0
45	First	3	0	0
	Second	2	0	0
46	First	4	0	0
	Second	4	0	0
48	First	4	0	0
	Second	4	0	0

animals comprised in each herd. In practice, during the absence, as now, of epidemic foot-and-mouth disease, the system is useless, but the machinery at present in existence can undoubtedly be readily utilised for that efficient system of registration of milch goats that the Local Government will be compelled to enforce if it seriously desires to stamp out Malta Fever from the island.

Table V.—Third Examination of Certain Herds at Rabato.

No. of herd.	No. of examination.	No. of goats in herd.	No. of milks reacting.	No. of milks containing *M. melitensis*.
14	First	5	3	2
	Second.........	3	1	0
	Third	5	0	0
18	First	21	6	0
	Second.........	21	0	0
	Third	21	2	1
35	First	4	0	0
	Second.........	4	0	0
	Third	4	0	0
47	First	9	1	0
	Second.........	11	0	0
	Third	11	0	0

A further point, and one of considerable practical importance, depends upon the inconsistent results obtained at succeeding examinations, and is brought out in Table V. Taking the herds in order, at the first examination of Herd No. 14, which comprised five milch goats, the milk from three was found to give an agglutination reaction, and from two of these the micrococcus was recovered. These last two animals were removed to the Lazzaretto. At the next examination of the three remaining animals the one that had previously given a positive agglutination reaction still reacted, but the milk did not contain the coccus. At the third examination the owner had added two more milch goats, and none of the five reacted, that is to say, the reaction had disappeared from the animal that had reacted on two previous occasions.

Herd No. 18 consisted of 21 goats, including six whose milk gave a positive reaction. At the second examination none of these six reacted, while at the third examination two of those which had previously reacted again showed the reaction, and the milk of one of them now contained the specific coccus.

Herd No. 35 consisted of four healthy milch goats, none of which showed any sign of infection at the subsequent examinations.

Herd No. 47 consisted at first of nine goats, and the milk from one of them gave a positive agglutination reaction at the first examination, but a negative reaction at the second and third examinations. Subsequently the owner added two healthy animals to his herd.

These results show conclusively the necessity for repeated examinations at short intervals of every herd if the detection of *all* the infective animals is aimed at.

2. *The Agglutination Test as Applied to the Milch Goat.*

The possibility that the Government of Malta might at some future period, should the infectivity of goats' milk be conclusively established, legislate on the subject, was fully appreciated in planning the observations on the milk supply of Rabato, and methods of examination were carefully investigated with a view to simplifying and systematising the routine analyses of milk that would have to be undertaken by the Public Health Department in such a contingency.

In the first place, there is no evidence that the ingestion of specific agglutinins with the milk is, *per se*, injurious to health, and the legislature would undoubtedly require the recognition of the *M. melitensis* itself in the milk as proof that such milk was noxious in character; and as in the administration of any regulation dealing with milk· the frequent examination of a large number of animals would have to be undertaken, it became necessary to determine the simplest, most rapid, and most reliable method of sorting out the animals which were most likely to be passing the micrococcus in their milk before proceeding to attempt the isolation of the micro-organism by bacteriological methods.

Such a method already existed in the milk agglutination test devised by Zammit and bearing his name, a test which depends upon the fact that *M. melitensis* agglutinins, like other specific agglutinins and antibodies when present in the body in sufficient quantity, pass into the milk, and to a less extent into the urine, and can be readily demonstrated in these situations. This test, it must be stated, has limitations which form its strong feature for the purpose with which we are at present concerned.

The work carried out by the Commission in 1905 showed clearly that in presumably very remote or in very recent infections the presence of agglutinins could not be demonstrated in the milk, although they were present in the blood in sufficient quantity to yield a positive reaction in low dilutions when the blood serum was tested. On the other hand—and this is the point of practical importance—*M. melitensis* has never yet been isolated, in the present series of experiments, from a sample of milk from which the specific agglutinin was absent.

In its elemental form the test under consideration consists primarily of the admixture of equal parts of the suspected milk and an emulsion of *M. melitensis* in normal saline solution. As originally devised, it was completed in one of two ways, either by making a hanging drop preparation of the mixture and observing it microscopically after the lapse of 12 hours, or by running the mixture into capillary tubes or sedimentation pipettes, and observing macroscopically after a similar period.

A reference to a previous part of these Reports (IV, pp. 55 and 97—98) will show that a difference of opinion existed as to the best method of performing the test, some observers preferring the hanging-drop method, others the tube method. In point of fact, both had many disadvantages.

In the microscopical method, quite apart from the difficulty of entirely preventing evaporation when the period of observation continued for so long a time as 12 hours, the difficulties introduced by the presence of masses of varying sized oil globules, which obscured all but the very large clumps, were considerable, and in the event of observations being made by partially-trained observers would undoubtedly lead to inaccurate results. These considerations early led to the rejection of this form of the test as a practical measure.

The macroscopical method was next studied, and as the perfect emulsification of the fat which obtains in goats' milk was undoubtedly the cause of the disfavour with which the test was received, a low dilution of 1 : 3 was first employed. In this series of tests numerous observations were made, carefully checking the naked eye results by running the deposit out of the capillary tube or sedimentation pipette and examining it microscopically. It was soon found that when results recorded at five hours were taken this method was a satisfactory and reliable one, but when tubes were left standing overnight the results noted in the morning were as often as not fallacious owing to the formation of a bulky deposit consisting of *débris*, leucocytes, etc., and not of clumps of *M. melitensis*.

Next was tried the effect of first converting the milk into curds and whey by the addition of rennet or of acid, separating off the curds and testing the whey. A number of experiments were made simultaneously with various samples of milk, and with whey prepared from each sample, and it became at once apparent that the agglutinins, unaltered, were present in the whey. The whey reaction was given equally well, was as definite as with clear serum, and obviously no confusion could arise with regard to pseudo reactions, due to the collection of *débris*, so that apart from the extra time and labour involved in preparation, the whey gave eminently satisfactory results.

On subsequently plating, however, it was found that cultivations prepared from whey yielded only about 10 per cent. of the number of micrococci developing from the corresponding milk sample after an identical period of incubation, a result possibly due to retention of cocci in the curd, possibly to destruction of cocci by acid.

Further dilutions of the milk itself were then tried, and finally it was determined that the most trustworthy and convenient method was to employ a dilution of 1 : 20 in capillary tubes or sedimentation

Table VI. —Comparison of the Agglutination Value of Milk

Date	July, 21, 1906.			July 24, 1906.			July 26, 1906.			July 28, 1906.		
Dilution	1 : 20	1 : 50	1 : 100	1 : 20	1 : 50	1 : 100	1 : 20	1 : 50	1 : 100	1 : 20	1 : 50	1 : 100
Goat 101—												
Milk	0			+	+	—	0			+	—	—
Serum				+	+	+				+	+	+
Goat 102—												
Milk	0			—	—	—	0			+	+	—
Serum				+	+	+				+	—	—
Goat 103—												
Milk	0			—	—	—	0			—	—	—
Serum				+	+	+				+	+	+
Goat 104—												
Milk	0			+	—	—	0			+	—	—
Serum				+	—	—				+	+	+
Goat 105 —												
Milk	0			+	+	+	0			+	+	—
Serum				+	+	+				+	—	—
Goat 106—												
Milk	0			+	+	+	0			+	+	—
Serum				—	—	—				—	—	—
Goat 108—												
Milk	0			+	+	—	0			+	—	—
Serum				+	+	+				+	+	+
Goat 110—												
Milk	+	+	±	0			+	—	—	0		
Serum	+	+	+				+	+	+			
Goat 111—												
Milk	+	+	±	0			+	±	±	0		
Serum	+	+	±				+	+	+			
Goat 112—												
Milk	+	+	+	0			+	±	±	0		
Serum	—	—	—				—	—	—			
Goat 113—												
Milk	+	+	+	0			+	+	+	0		
Serum	+	+	+				+	+	—			
Goat 114—												
Milk	+	±	±	0			+	+	±	0		
Serum	+	+	+				+	+	+			
Goat 115—												
Milk	+	+	—	0			+	+	+	0		
Serum	±	±	—				+	—	—			
Goat 117—												
Milk	+	+	—	0			+	+	+	0		
Serum	+	+	—				+	—	—			
Goat 118—												
Milk	+	—	—	0			+	+	—	0		
Serum	+	—	—				+	+	—			
Sheep 107—												
Milk	0			+	+	—	0			+	—	—
Serum				+	+	+				+	+	+

0 = not examined.

and Blood Serum in the Infected Milch Goat and Sheep.

July 31, 1906.			August 2, 1906.			August 4, 1906.			August 7, 1906.		
1 : 20	1 : 50	1 : 100	1 : 20	1 : 50	1 : 100	1 : 20	1 : 50	1 : 100	1 : 20	1 : 50	1 : 100
0			+ +	— +	— +	0			+ +	+ —	— —
0			— +	— +	— —	0			+ —	± —	± —
0			— +	— +	— —	0			+ +	+ +	— +
0			+ —	— —	— —	0			± +	± +	— +
0			+ +	— —	— —	0			+ —	+ —	— —
0			+ +	+ +	— +	0			+ —	— —	— —
0			+ +	— +	— +	0			+ +	+ +	+ +
+ +	— +	— +	0			+ +	+ +	+ +	0		
+ +	+ —	± —	0			+ +	+ +	± +	0		
± —	— —	— —	0			+ +	— —	— —	0		
+ +	+ +	+ +	0			+ +	+ +	+ +	0		
+ +	— +	— +	0			+ +	+ +	+ +	0		
+ +	± +	± +	0			+ +	+ +	+ +	0		
+ +	— —	— —	0			+ +	+ +	— +	0		
— +	— +	— +	0			+ +	— —	— —	0		
0			± +	— +	— —	0			+ +	+ +	+ +

0 = not examined.

pipettes with a time limit of 24 hours—a method carried out in actual practice by mixing equal volumes of a 1 : 10 dilution of milk with the previously prepared emulsion of *M. melitensis* in distilled water. Under these conditions the fluid is sufficiently clear to show the reaction quite as definitely as in the case of blood serum, while the specific gravity is so altered that the fat globules are very quickly disentangled and rise to the upper part of the column of fluid as cream, and the risk of pseudo reactions from sedimentation of *débris* is avoided.

By repeated experiments it was found that where a true reaction was yielded by the low dilution method (1 : 3), a reaction was obtained in 1 : 20 dilution; but on carrying the dilution higher it was found that milks giving a positive reaction in 1 : 3 and in 1 : 20, and which on plating yielded *M. melitensis* in some instances, gave a negative reaction in 1 : 50.

Moreover, it was found that in the case of sheep the low dilution (1 : 3) did not give reliable results owing to the opacity of the milk; whilst with a 1 : 20 dilution consistent and reliable results were obtained.

The test as thus modified has now been in use for many months, and the best criterion of its utility is summed up by the fact that *M. melitensis* has never been isolated from or detected in a sample of milk which yielded a negative reaction.

It was also noted during the course of these experiments as a general, but far from invariable, rule, that those milks which gave a good sedimentation reaction immediately upon mixing with the bacterial emulsion, or within an hour or two, were more likely to yield cultivations of *M. melitensis* on plating than those which required the full 24 hours for the completion of the reaction.

A comparison of the milk agglutination reaction with the blood agglutination reaction of the infected goat naturally formed part of these experiments with the "Zammit test."

The milk and blood samples were collected at the same time, the test applied with the same bacterial emulsion, and identical dilutions of 1 : 20, 1 : 50, and 1 : 100 were employed in each case.

Roughly speaking, the reactions are comparable: on the whole the blood frequently reacts in higher dilutions than the milk, though in some instances the opposite holds good. In two animals (Goats Nos. 106 and 112) the milk reacted in 1 : 20 when agglutinins appeared to be absent from the blood; in a third (Goat No. 103) the converse obtained, the milk did not react in 1 : 20, while the blood did so. No relationship whatever could be deduced between the intensity of the reaction and the numbers of *M. melitensis* present in the milk. A number of these observations are tabulated on pp. 16 and 17.

The table shows, too, in a most marked manner, the periodic variation in the agglutination value of the serum, the curve of which is closely followed by that of the milk, due no doubt to auto-inoculation or auto-vaccination with living *M. melitensis* taking place in milch goats which are still the subject of a subacute systemic and generalised infection—an explanation which accords well with observed facts, and which is supported by the results of observations made upon milch goats which were under treatment with vaccine prepared from dead cultures of *M. melitensis*.

3. *The Numerical Relationship of* M. melitensis *to the Milk of Infected Goats.*

As it had already been noted that the milk from an infected goat varied greatly from day to day in its potentialities for evil—that is to say, milk which contained many thousands of individual *M. melitensis* per cubic centimetre when examined one day, at the next examination a few days later, or even on the following day, might be apparently free from the micro-organism, and again in a few more days might be crowded with the cocci—systematic observations were commenced to determine, if possible, whether some definite periodic variation, seasonal or otherwise, existed. These observations are as yet incomplete, but the results of nearly four months' work are available, and on account of their interest are inserted here.

The results show that although the excretion of *M. melitensis* in the milk during some stages of the infection is persistent, it is by no means constant or even consistent; nor—premising that all the observations now recorded were carried out during the so-called summer—was it possible to determine any correlation between the temperature curve and the number of cocci excreted in the milk (*vide* Fig. 2, p. 24). The observations which are being continued throughout the winter may, however, afford further information in this connection when completed. The animals affording material for the enquiry were comprised in a herd of 17 milch goats and 1 milch sheep belonging to the Commission and stalled in the Lazzaretto. Twice and often three times a week a specimen of milk was collected from each animal, immediately conveyed to the Laboratory at Valletta, and there plated out. On account of the extreme variation noted in the numbers developing per cubic centimetre from each animal's milk, together with the necessity for examining the milks from a number of animals each day, it was a matter of some difficulty to decide on the amount of milk to be plated for each examination. After many experiments in this direction 0·035 c.c.—an amount equivalent in bulk to a single drop falling by gravity from the end of a fine capillary pipette—was decided upon, and three plates were inseminated, each with this quantity of milk, from every goat at each examination. In plating, the milk was deposited on the centre of a nutrose-agar plate and distributed all over the surface by means of a sterile L-shaped glass rod spreader. After suitable incubation, the colonies of *M. melitensis* that had developed on each of the three plates were enumerated and averaged, the contents per cubic centimetre calculated and the results obtained recorded in tabular form, and also plotted in a curve.

Table VII.—Numerical Strength of *M. melitensis* per cubic centimetre in Milk of Infected Goats.

Serial No. of goat	104.	105.	106.	107.	111.	112.	114.	115.	117.
1906—									
May 7...	4,000	nil	330	7,000	—	—	—	—	—
,, 10...	800	**30,000**	200	?	15,000	100	—	—	—
,, 15...	13,000	nil	nil	2,400	6,000	nil	—	—	—
,, 22...	**30,000**	nil	nil	6,000	**30,000**	nil	—	—	—
,, 26...	3,000	nil	nil	4,000	6,000	4,000	—	—	—
,, 29...	30	nil	nil	4,600	15,000	nil	—	—	—
,, 31...	nil	nil	nil	6,500	**30,000**	100	nil	6,000	8,000
June 2...	66	nil	nil	10,000	5,700	nil	**30,000**	15,000	8,000
,, 5...	7,500	nil	nil	6,000	12,000	30	4,300	**30,000**	**30,000**
,, 9...	nil	nil	nil	nil	20,000	nil	?	4,600	5,000
,, 12...	830	nil	nil	7,300	3,400	66	nil	**30,000**	**30,000**
,, 14...	nil	5,000	nil	10,000	8,000	100	1,760	5,500	**30,000**
,, 16...	66	nil	nil	500	6,000	30	14,000	100	nil
,, 19...	nil	nil	nil	nil	**30,000**	700	700	430	?
,, 21...	30	nil	nil	30	2,400	2,000	nil	23,000	1,200
,, 23...	130	nil	2,000	5,000	2,700	3,200	1,500	660	6,666
,, 26...	66	nil	2,000	15,000	13,000	**30,000**	20,000	1,000	13,000
,, 28...	200	nil	nil	**30,000**	12,000	2,600	15,000	2,500	20,000
,, 30...	1,500	30	400	**30,000**	23,000	1,000	18,000	4,700	23,000
July 3...	1,100	nil	330	3,000	6,170	66	16,000	8,000	**30,000**
,, 5...	400	nil	30	2,400	4,000	66	20,000	17,000	27,000
,, 19...	700	10,000	nil	23,000	—	—	—	—	—
,, 21...	—	—	—	—	730	nil	15,000	**30,000**	**30,000**
,, 24...	470	8,300	nil	2,300	—	—	—	—	—
,, 26...	—	—	—	—	1,000	nil	4,000	20,000	23,000
,, 27...	—	—	—	—	4,000	—	800	300	1,000
,, 28...	66	nil	nil	1,700	—	—	—	—	—
,, 31...	—	—	—	—	30	nil	**30,000**	1,700	16,000
Aug. 2...	16,000	1,200	nil	1,800	—	—	—	—	24,800
,, 4...	—	—	—	—	2,700	nil	3,000	730	nil
,, 7...	660	5,300	100	1,000	—	—	—	—	—
,, 14...	1,600	nil	540	20,000	nil	nil	nil	8,000	**30,000**
,, 18...	750	6,600	nil	720	10,000	nil	6,000	nil	nil

— = not examined.

In connection with this table a fallacy and an inaccuracy must be pointed out. In the first place, the fallacy consists in recording *nil* per cubic centimetre, when only three separate small amounts of milk —totalling in the aggregate but a little more than 0·1 c.c.—proved to be sterile or had failed to give rise to a single colony of *M. melitensis.*

Such a result, however, is amply sufficient to show that the *M. melitensis* content of the milk is extremely small, and, bearing this fallacy in mind, it is sufficiently accurate for the purposes of a comparative table to record such a result as *nil.* The inaccuracy relates to the other extreme. In enumerating colonies on a plate it is usual to count each individual colony, when the number does not exceed 1,000, by the help of radii drawn on the bottom of the glass dish with a grease pencil. Beyond this figure it is customary to observe the colonies by the aid of a low power objective, to enumerate those enclosed in several areas of the plate, and average the contents of such areas. For this purpose a 1-inch or a $\frac{2}{3}$-inch objective, a suitable eye-piece and such particular length of tube is employed as will produce a field whose area bears a definite relationship to the total area of the plate, so that finally the approximate content of the entire plate is rapidly calculated from the content of an average field. The recorded figures between 100 and 1,000 may therefore be regarded as accurate; figures in excess of 1,000 calculated from the average contents of selected microscopical fields are probably highly inaccurate; but as in all these estimations care was taken to select the fields for counting from the least crowded portions of the plates the inaccuracy was always in the direction of underestimation. In some instances so obvious was the underestimation that 30,000, the highest figure recorded—as it was necessary to keep the curves that are presented herewith (see Fig. 2) within reasonable limits—is indicated in special type to show that, in the opinion of the observers, at least one other zero should be added, as the numbers were practically uncountable.

This difficulty in enumeration will be better appreciated by a glance at Fig. 1, which represents a plate inoculated with 0·035 c.c. of milk from Goat No. 111. With the exception of the few large colonies of saprophytic bacteria, all the colonies are due to the growth of *M. melitensis.* The milk from which this plate was prepared is returned as containing **30,000** *M. melitensis* per cubic centimetre.

On several occasions control plates were prepared with suitable dilutions of the milk samples, and it was frequently found that 3,000,000 per cubic centimetre would be much nearer the true content of the milk than the 30,000 recorded.

In this connection it may be remarked that the conditions under which work with goats' milk is carried out in Malta are totally different from those that hold in England, when the corresponding food, cows' milk, is being investigated. It must be obvious to those who are in the habit of working with cows' milk at home, that the enumeration of colonies of any specific organism, *e.g.*, streptococci, in plates prepared by smearing milk direct upon the surface of the medium, would be a hopeless task on account of the number of extraneous saprophytes present. With the Maltese goat, however, having discarded the first few cubic centimetres of fore milk, the next quantum, the mid milk and the strippings, consist of sterile, or practically sterile, milk, in the case of the normally healthy milch goat, or a pure culture of *M. melitensis* in the case of the milch goat which is infected by *M. melitensis* and is voiding the germ in its milk. Some of the reasons for the clean character of the milk are fairly patent, although the goat is an extremely dirty animal in its choice both of food and resting-place. Most important of all is the fact that the milk is examined within a very short time of its removal from the mammary gland of the goat—no long interval, occupied in transit by road and rail, is allowed for the multiplication of extraneous saprophytes. Again, in milking, the goatherd invariably crouches

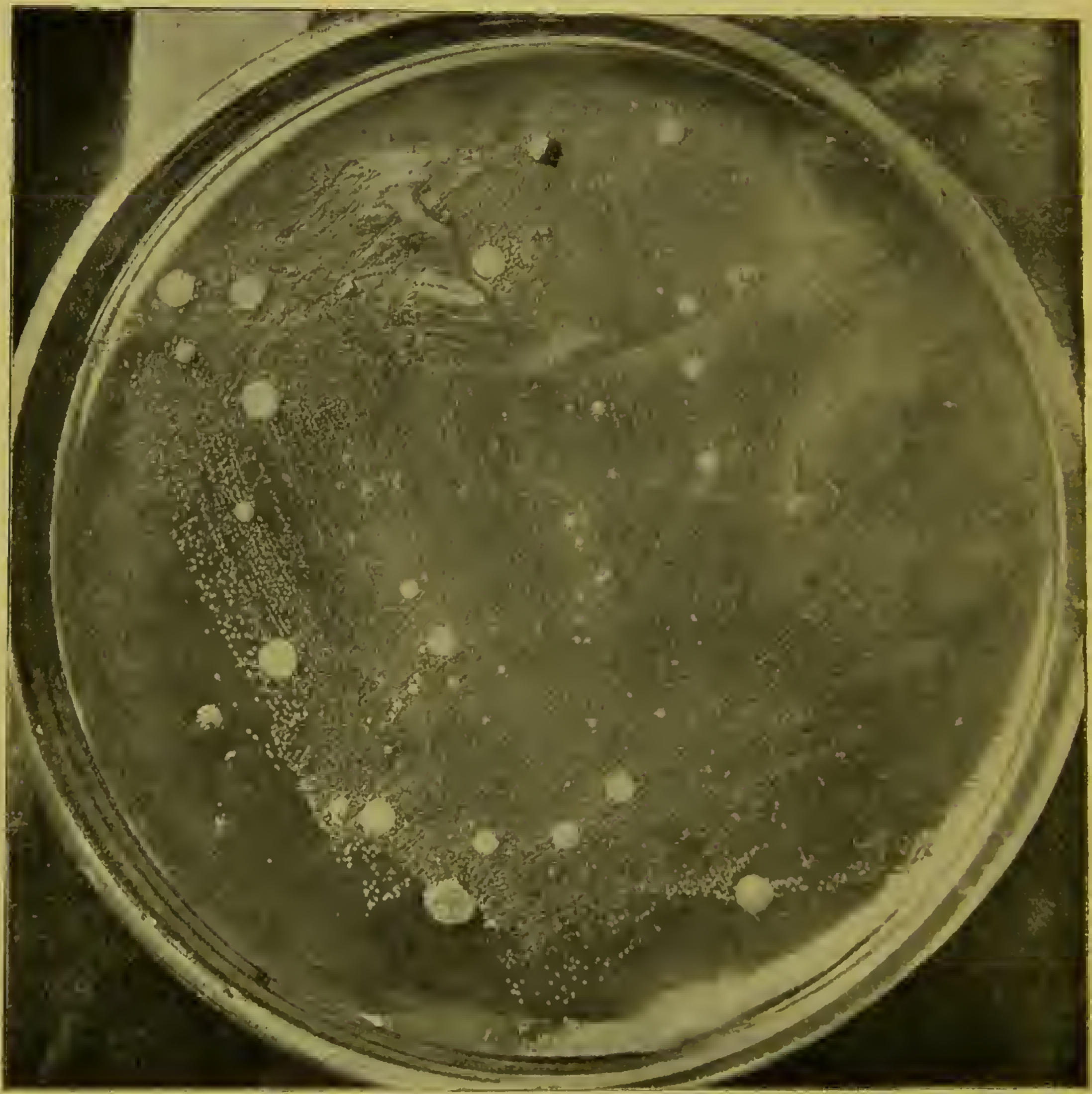

FIG. 1.—Nutrose agar plate prepared from 0·035 c.c. freshly drawn milk from Goat 111. All the minute colonies are composed of *M. melitensis*.

directly behind the animal, and grasping the udder, drags it back between the hind legs, which the animal separates widely to allow of its passage. The receptacle for the milk, if small, is held in one hand some distance behind the animal and below the level of the teat, while the other hand, grasping udder and teat, directs an oblique stream of milk into it—in other words, the jet of milk and the receiving vessel hold the same relative positions as the inoculating platinum needle, and the test-tube respectively, when a tube culture is being prepared in the laboratory. If the receptacle for the milk is large, it is placed on the ground behind and well clear of the goat, and the goatherd, employing both hands, milks from both teats; consequently, any dirt and filth dropping from the udder and hindquarters falls more or less vertically to the ground, while the stream of milk traverses a stratum of clean air which does not usually add saprophytes to the milk during its passage; indeed, the freedom of the air and dust from saprophytic bacteria is a noteworthy feature of bacteriological research in Malta, and is due no doubt to the sterilising action of the direct rays of the sun—which are available for at least 12 hours per diem for the greater part of the year. Aerial contamination of cultivations in a laboratory kept moderately clean is rare, plate cultures can be made in the open air with a reasonable certainty that, where not intentionally infected, they will remain sterile, and plates may be opened and examined day after day without the preparations becoming contaminated.

For the moment the only explanation that can be offered of this day-to-day variation in the number of cocci present in the milk is that the micro-organism, lodged in a suitable soil and richly supplied with a medium of high nutritive value, multiplies rapidly in the interstices between and upon the surface of the gland epithelium cells. This multiplication proceeds up to a certain point, when, owing perhaps to the mechanical irritation set up by the mere presence of the coccus, a flushing process is carried out by the milk itself which removes the excess of cocci and leaves behind in the gland tissue only those cocci which are in intimate relationship with the gland cells. A certain interval necessary for the further multiplication of those cocci left behind elapses, then the process is again repeated, and so on.

Conversely, it is more than probable that the stimulus provided by the presence of the bodies of the cocci, or of their toxins, is an important factor in the large and long-continued milk yield of the infected milch goat.

In the accompanying Graph, the numbers of *M. melitensis* obtained at the periodic examinations of the milk from Goats 111, 114, 117 are plotted out in curves superposed on the curve representing the diurnal mean temperature of the air of Valletta. On the one hand, it will be seen that there is no direct association between discharge of cocci in the milk and rise or fall of air temperature; on the other, the

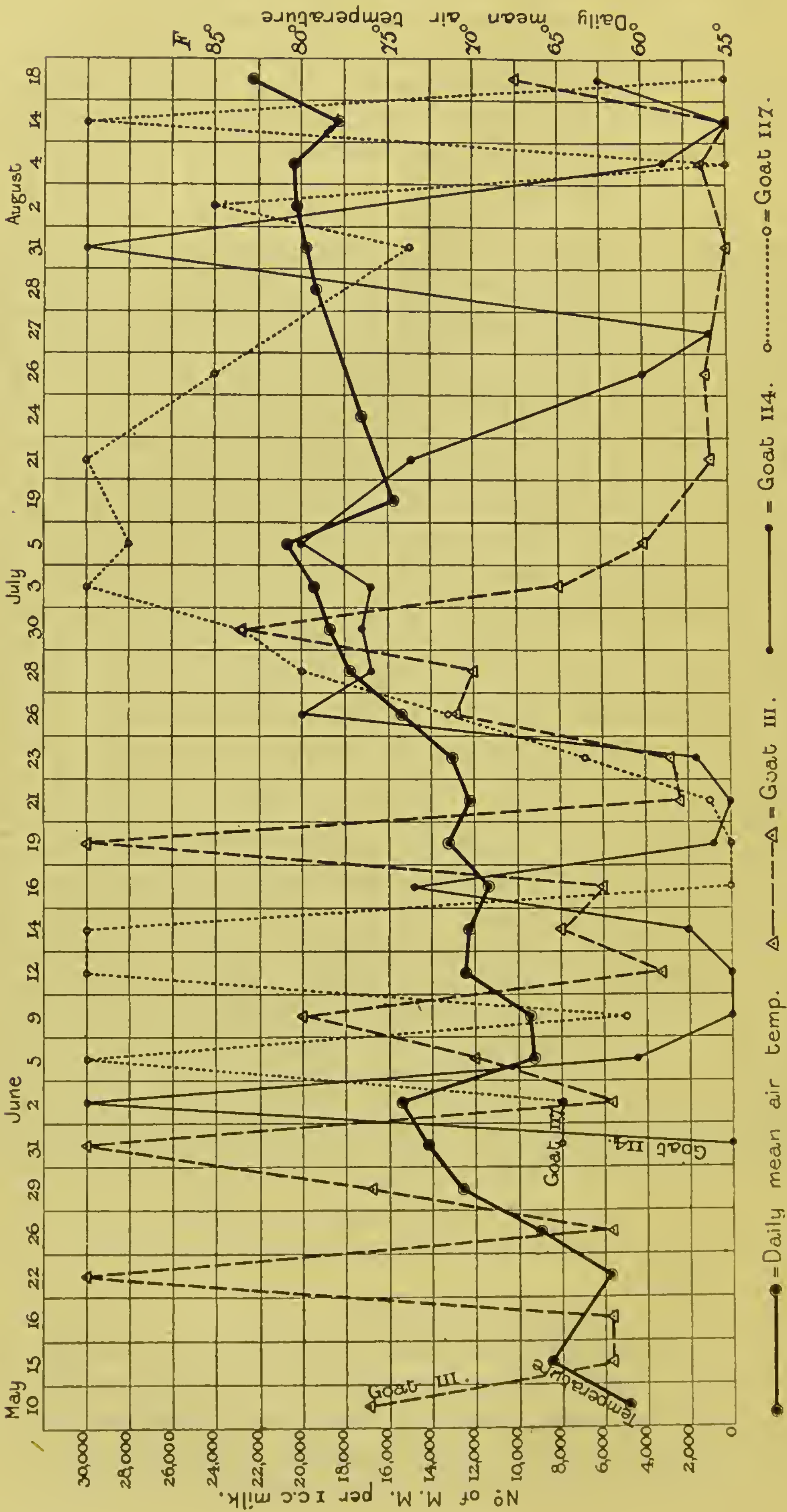

FIG. 2.—Curves showing the periodic variation in the *M. melitensis* content of milk of infected goats.

periodic discharge of large quantities of cocci and the variable interval during which but few cocci are present in the milk, are well brought out.

4. *The Presence of* M. melitensis *in Milk Products.*

The fact elicited as the result of the examination of a very large number of animals, that at least 10 per cent. of the milch goats in the Island of Malta secrete milk which contains the specific germ of Malta Fever in divers quantities, rendered it extremely likely that the micrococcus might pass unscathed through the various manipulations to which milk is subjected in the preparation of articles of food such as cheese, etc., and some observations were accordingly made in this connection.

Cheese.—The native cheese in common use is a whole milk cheese, and is prepared in a very simple and primitive manner. Acid or rennet is added to the sheep's or goat's milk, and the resulting curd is "set" in open basketwork moulds which allow the whey to drain off, placed aside overnight, and is ready for consumption the following day. Such a cheese is, of course, eaten fresh, as its method of preparation hardly fits it for prolonged storage.

At first sight it would appear probable that the high degree of acidity present in the cheese (a fair average would be + 80 or + 90 as compared with an optimum of + 8 or + 10 aimed at in preparing nutrient media for the cultivation of *M. melitensis*) would ensure the destruction of the micrococcus. A reference, however, to the work of Shaw* shows that the micrococcus can retain its vitality in urine, having a reaction of + 40, + 50, or + 60; and, again, it was noted in carrying out certain experiments as to the presence of agglutinins and of the specific coccus in whey, prepared from infected milk, that the coccus retains its vitality in this highly acid whey, while the diminution in number in this whey as compared with the original milk would suggest that numerous cocci remain entangled in the curd, quite as much as that numerous cocci are killed off by the acid present.

The first sample of cheese to be examined was obtained 24 hours after setting, direct from its manufacturer, a peasant farmer near Zebbug, who kept a herd of 13 milch goats and one milch sheep. (Seven of his goats, the sheep and a sow were subsequently found to be infected, and the goats and sheep were secreting milk containing *M. melitensis*. These animals, purchased by the Commission and lodged in the Lazzaretto, afforded material for many of the observations recorded in the previous pages.)

Plate cultivations in series on the surface of nutrose-agar were established in the usual way from pieces cut from the centre of the cheese with a sterile knife, after well searing the surface with a red-hot iron: and also from 0·1 c.c. of an emulsion prepared by breaking up approximately 0·1 gramme of cheese in 10 c.c. of sterile broth. Three plates only were prepared in each series, and after incubation it was found that the dilution was insufficient, as all were crowded with saprophytic bacteria which rendered the "fishing" of the colonies of *M. melitensis*, which were recognised with the hand lens, a matter of impossibility. Scrapings from the plate, which included colonies of the coccus as well as neighbouring saprophytes, when emulsified in distilled water and added to a 1 in 250 dilution of specific serum, showed microscopically typical clumps of *M. melitensis*. Ordinary sub-cultures failed to give a growth of the micrococcus, and as it was considered waste of time to continue work on this cheese, the plates were abandoned and further experiments made with cheeses prepared in the Lazzaretto from the milk of the identical infected sheep and goats—now the property of the Commission—from which this first specimen had been manufactured.

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* These Reports, III, p. 43 *et seq.*

Numerous cheeses were made and each examined repeatedly during several days, and it was found that by careful attention to the question of dilution in preparing the series of plates, it was a fairly easy matter to isolate the coccus from the cheese up to the end of 48 hours. After this time the multiplication of lactic acid and other bacteria outstripped that of *M. melitensis*, and it was no longer possible to recover the organism.

Ice Cream.—With the advent of hot weather, soon followed by the discovery that the ordinary private soldier does purchase ice-creams in the local cafés and consume them, attention was directed to this milk product. Enquiry elicited the fact that, as in England, two varieties of ice-cream are in vogue, the one prepared by freezing a flavoured custard of milk and eggs previously heated to about 80° or 90° C. and cooled, the other by flavouring milk to which cream may or may not be added, with various essences or syrups and freezing—the former being the more expensive. As by this time the infectivity of goats' milk was becoming a matter of common knowledge among the Restauranteurs of Malta, one individual volunteered the information that he always boiled his goats' milk before using it in the manufacture of ice-cream. On the other hand, it has been stated by a private in the Royal Army Medical Corps that a café keeper who had run out of his stock of ices would milk the goat tied up to his door post, and some 20 minutes later produce the ice-cream to his customer.

Experimental ice-creams of both types were, therefore, prepared in the laboratory from milk derived from the infected herd at the Lazzaretto, and then examined for the presence of the *M. melitensis*. In the case of those made from a custard of milk, eggs, and sugar, and heated to about 80° C. to ensure the thorough incorporation of the ingredients, it was found impossible to recover living *M. melitensis*, although it was known they were present in abundance in the infected milk—from the result of plate cultivations established prior to heating. This was only to be expected, as the thermal death point of the organism in watery suspension has been determined as 57·5° C.

In the case, however, of the second type of cream where no heating is resorted to, it was found to be as easy to recover *M. melitensis* from such as had been prepared from infected milk as it was from the milk itself.

With these data to work upon, a series of examinations of samples of ices from various cafés was instituted. The ice-cream, on arrival in the laboratory, was placed in the incubator at 37° C. for an hour or two to thaw. The ortol and peroxide of hydrogen test* was next applied to determine whether or no the milk had been boiled or had been heated above 70° C., although often ocular observation was sufficient in the case of such as had been prepared from custard. In the event of the sample of cream having been made from custard, the specimen was, of course, discarded, but if it responded to the test and gave the characteristic reaction associated with unheated milk, then plate cultivations in series were established from the cream and set aside in the incubator. Some of the fluid portion of the ice-cream was diluted to 1 in 10, with sterile water added to an equal quantity of emulsion of *M. melitensis*, taken up in sedimentation tubes and set aside for observation as to the presence or absence of agglutinins.

If, on the following morning, the sedimentation reaction was absent, the plates made from that particular ice-cream were discarded, as previous experiments in this connection (p. 18) had shown that *M. melitensis* was never recovered from milk in the absence of a good agglutination reaction in dilution of 1 in 20.

In all, 11 samples of retail ice-creams were examined before illness interfered with the investigation. Three had been prepared from heated custard, and were consequently discarded; four others, prepared from unheated milk, were plated out, but as on the following morning the sedimentation reaction was absent, these plates were not proceeded with. The four remaining samples—made from unheated milk—yielded a complete sedimentation reaction, and the plates prepared from them were carefully worked out, but with negative results; *M. melitensis* could not be detected.

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* This is a test introduced by Saul some few years ago, and depends upon the fact that the addition of ortho-methyl-aminophenol sulphate (or of ortol, an impure preparation of the same salt, used chiefly in photographic work) to raw milk in the presence of nascent oxygen, gives rise to a brick-red coloration within 30 seconds of mixing. With milk which has been heated at or above 70° C., no change is observed.

In practice the test is carried out by adding 1 c.c. of a freshly prepared aqueous solution of ortol (1 per cent.) to 10 c.c. of milk in a test-tube and then adding one or two drops of hydrogen peroxide (3 vols.).

Further investigations in this direction are now in course of prosecution.

Butter.—As practically all the butter consumed in the island is imported tinned butter and margarine, no examinations of samples of this food were made.

5. *Susceptibility of the Goat to, and Paths of Infection by* M. melitensis.

As the results of the examination during the years 1905 and 1906 of a very large number of animals scattered throughout the island had revealed the fact that, roughly, some 10 per cent. of the milch goats were secreting milk containing the coccus, while some 30 per cent. more by the existence of fair quantities of specific agglutinins in the blood serum and milk afforded the strongest presumptive evidence of previous infection, it is obvious that the goat is distinctly susceptible to the pathogenetic influence of *M. melitensis.* The experimental work carried out during the same years also showed clearly that the goat could be infected, with a fair amount of ease, by the ordinary laboratory methods of inoculation—that is by the subcutaneous and intravenous injection of living cultivations of *M. melitensis*—and also by feeding, either with artificially or naturally infected material.

These observations rendered any extended series of experiments in similar directions needless; on the other hand, no experimental results were available with regard to infection by contact, such as had already been shown took place in the case of monkeys in close proximity to each other.*

Contact Infection.—Two yearling female goats—Nos. 22/5 and 23/5—which had not yet been impregnated, and consequently were not "in milk," and from whose blood specific agglutinins were absent, were, therefore, turned loose into a large stable in company with 16 infected milch goats and one infected milch sheep, from all of whose milk *M. melitensis* was obtainable in varying numbers. None of the animals were tethered; consequently the contact was as intimate as possible, while the fact that neither of the two experimental animals were included in the daily milkings eliminated the likelihood of transmission of infection viâ the goatherds' hands. Eight days after the commencement of the experiment the blood serum of Goat No. 23/5 yielded a partial agglutination reaction in a dilution of 1 in 10; on the following day the serum from Goat No. 22/5 gave a similar result, and on the 16th day both goats gave a complete positive agglutination reaction in dilutions 1 in 10 and

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* *Vide* these Reports, I, p. 67.

1 in 20. Subsequent examination of the blood serum from each goat showed those fluctuations of the agglutination curve that are usually associated with *M. melitensis* infections, and the serum value six weeks after the commencement of the contact reached 1 in 100. At the end of four and a-half months both goats were killed and examined *post-mortem*. The organs that were examined after death were spleen, inguinal glands, mesenteric glands, and kidneys, and from none of these was *M. melitensis* recovered. This failure to detect the specific micro-organism *post-mortem* in the case of infected goats has by no means the same significance that it would have in other experimental animals, a point which will be referred to in a succeeding section.

In this experiment infection may have taken place through actual contact, the ingestion of fodder saturated with infective milk or urine, convection by ordinary flies, transmission of the coccus by means of biting flies, etc., the conveyance of infective material on the goatherds' hands alone being eliminated.

Of the various methods above mentioned, it may be pointed out that some would be excluded under ordinary circumstances. For instance, in the native goat-house the goats are usually tied up on either side of a raised trough containing food, from which few or many feed in common, by a length of rope sufficient to permit of the animal lying down. Each animal retains the same position from day to day; consequently contact in the stable is limited to the neighbouring goats on either side, and when the troughs are arranged in rows to the animal immediately behind also, and it is a noteworthy fact that in many cases it is either the next-door neighbour or the goat immediately behind the infected goat that next becomes infected, and not the goat on the opposite side of the trough. When taken out of the goat-house to start on the daily milk round, contact is more promiscuous, though here, again, stable companions are said to keep together.

Subcutaneous Infection.—It next appeared desirable to determine whether comparatively small doses of living cocci from artificial cultivations could be depended upon to produce infection. For this purpose four normal, healthy, young kids were selected, and when repeated observations had shown that specific agglutinins were absent from the blood, they were injected subcutaneously, each with a different sized dose of infective material.

The strain of *M. melitensis* employed for the experiment was a first sub-culture (from a single colony) of the coccus isolated from the milk of Goat No. 111—one of the infected milch goats under observation in the Lazzaretto.

The actual culture used was a 48-hours agar-tube growth. One loopful*

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* An ordinary agar slope cultivation would be equivalent, approximately, to 50 such loopfuls.

Table VIII.—Inoculation Experiments with the Goat.

No. of goat.	Sex.	No. of cocci injected.	Bulk of infective emulsion.	Method of inoculation.	Serum reaction.		Duration of experiment in days.	*Post-mortem* findings.					
								Value of serum.	*M. melitensis* recovered from—				
					Day of appearance.	Amount of dilution.			10 c. mm. of blood.	Inguinal glands. R.	L.	Mesenteric glands.	Spleen.
10	♂	10,000,000	c.c. 1·0	Subcutaneously at root of left ear.	7	1 : 20	47	1 : 200	—	+	+	—	+
9	♂	1,000,000	0·1		7	1 : 20	47	1 : 100	—	+	—	+	+
5	♀	100,000	0·01		17	1 : 20	46	1 : 20	—	—	—	—	+
4	♀	10,000	0·001		17	1 : 10	46	1 : 20	—	—	—	—	+

of this growth was emulsified in 10 c.c. of sterile saline solution, and the four goats were inoculated subcutaneously at the root of the left ear with 1, 0·1, 0·01, and 0·001 c.c. respectively of the emulsion. Portions of the remainder of the emulsion, after suitable dilution, were plated out and incubated. Enumeration of the resulting colonies showed that the emulsion contained 10,000,000 cocci per cubic centimetre.

Consequent upon these injections all four goats became infected :, the blood serum from the first and second yielding a positive agglutination reaction with 1 in 10 and 1 in 20 dilutions on the seventh day. Serum from the third did not give a positive reaction until the seventeenth day in these dilutions, whilst the fourth, although giving a positive reaction in 1 in 10 dilution, gave an incomplete reaction only with 1 in 20 on the seventeenth day, and not until the thirtieth day did the serum react well with 1 in 20 dilution.

From the clinical point of view there is little to record in connection with these inoculated goats. At no time during the course of the experiment did either of the goats appear to be ill—their coats were in good condition, no glandular enlargement was to be observed on palpation, and the animals fed well, as usual. The temperature was somewhat irregular, but in this respect none differed markedly from the healthy animals that served as controls, as will be seen from the temperature charts (*vide* p. 32) of the four experimental animals and the one of the normal animals in an adjoining stable that showed the least extensive excursions from the normal line (which for the goat averages, according to Damant,* 103·5—104° F.), and so served as a control.

All the animals were killed at the end of six weeks, and at the *post-mortem* inspection ample evidence of *M. melitensis* infection was available in the recovery of the organism from the spleen in all four goats, and in three of them from other organs as well. Details of this experiment are shown in tabular form (see Table VIII).

A point of some interest may be noted in this experiment with regard to the relationship of the size of the dose of infective material to the date of onset of signs of infection. Thus the two first goats, which received 10 and 1 million cocci respectively, gave evidence of reaction to the infection, by the appearance of specific agglutinins in the blood, within a week of inoculation and a full 10 days earlier than the other two goats which had each received less than a million cocci.

Cutaneous Infection.—The suspicion that had been aroused with reference to the probability of infection being carried from goat to goat by way of the goatherd's hands when soiled with infective milk was strengthened by the knowledge that such a comparatively small amount of infective material injected subcutaneously was sufficient to produce infection.

The technique adopted by the Maltese milker closely resembles

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* 'Journ. Physiol.,' Cambridge, 35, 1906 (Proceedings, v).

Chart No. 1.

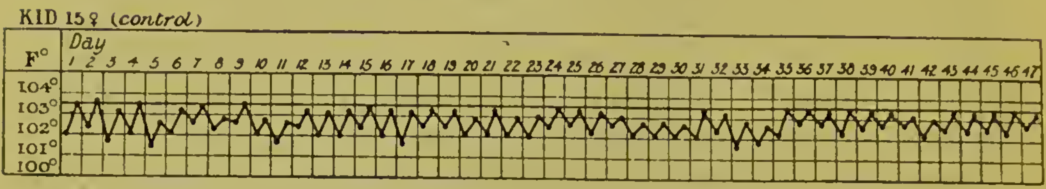


Chart No. 2.

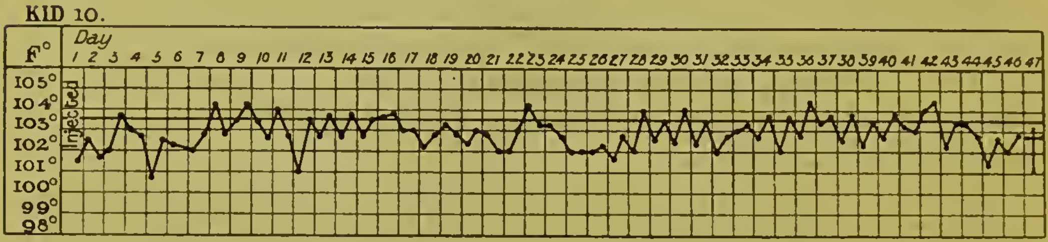


Chart No. 3.

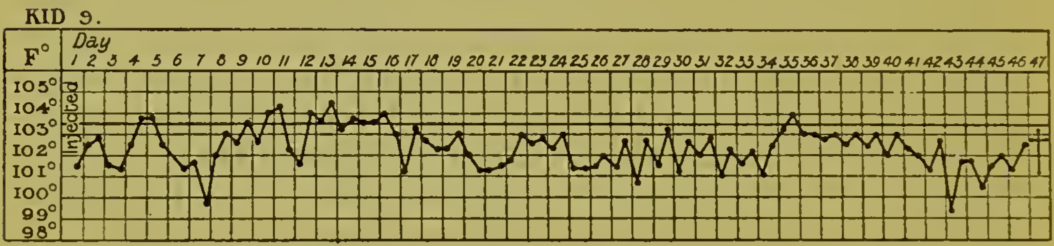


Chart No. 4.

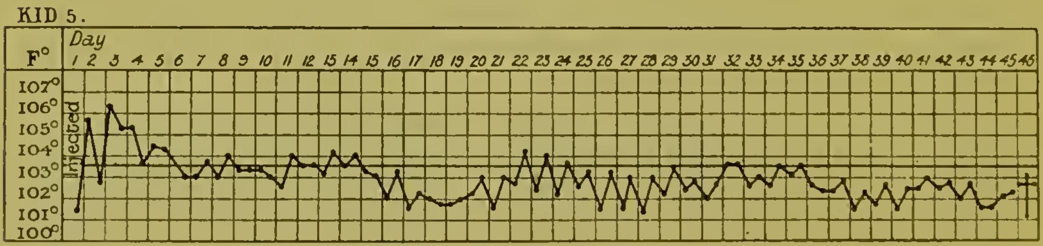
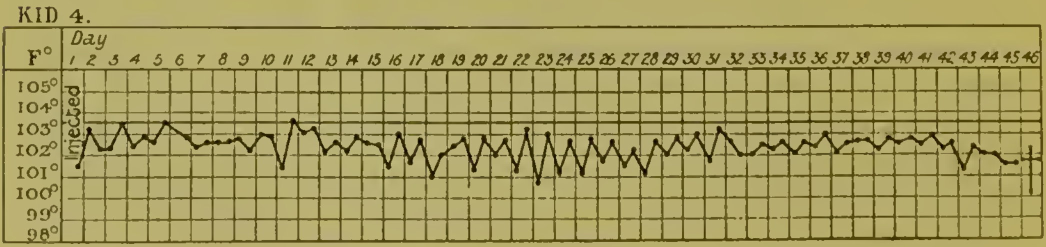


Chart No. 5.



Subcutaneous Infection of the Goat.

that of his English confrère, and consists in lubricating his own hands and the outside of the udder with some of the foremilk. When a number of goats have to be milked in rapid succession, the lubricant obtained from the first goat will serve for perhaps some half-dozen goats; with the seventh goat a fresh supply of milk is taken for the same purpose, and so on. Now, given that Goat No. 1 or Goat No. 7 is passing *M. melitensis* in its milk, it is obvious that, at any rate, Goat No. 2 or No. 8, as the case may be, stands a very good chance of becoming infected by a process of subcutaneous inoculation; consequently steps were taken to investigate the possibility of this occurrence.

In carrying out this experiment, the procedure of the goatherd was imitated as closely as possible, and Goat No. 25/6, a healthy female, nearly full grown, was selected for the purpose of the experiment, cast on an operating table and securely held by assistants. A fairly large area of skin over the left mammary gland was shaved somewhat roughly, in such a manner as to remove in many places the superficial layer of epithelium as well as the hair, but care was taken to avoid drawing blood. Thus the shaved area replaced the scratches, abrasions, and small ulcers that are so frequently seen in the udder and teats of the milch goat.

Next, the hands of the operator being protected by a pair of sterilised indiarubber gloves, four drops of freshly-drawn milk from Goat No. 117 (amounting in total bulk to 0·2 c.c.) were delivered into the palm of the right hand from a sterile capillary pipette, and then thoroughly rubbed into the shaved area with movements similar to those practised by the goatherd as a preliminary to milking. The quantity of milk used was so small that the skin surface rapidly dried, and the goat was then isolated in a stall apart from the other animals. Immediately after the experiment was concluded, a sample of the milk that had been used was carefully plated out (after suitable dilution) and found to contain 24,800 *M. melitensis* per cubic centimetre; the approximate number of cocci, therefore, that came into contact with the prepared area of skin amounted to 5,600.

Samples of blood were taken from a vein in the ear of this animal and examined from day to day for the presence of specific agglutinins, which first made their appearance on the fifteenth day (dilutions 1 in 10 and 1 in 20).

Three weeks after inoculation the goat was killed and a careful *post-mortem* examination carried out, with the result that the specific organism was recovered from the spleen and inguinal glands.

At the same time Goat No. 23/6, a four-month-old female kid which appeared quite healthy, was selected as a control. The skin over the mammary gland was carefully examined with a hand lens, and an area quite free from scratches, cracks, or fissures was isolated by painting flexile collodion on the surrounding skin. Eight drops of milk from

Table IX.—Cutaneous Inoculation of the Goat with *M. melitensis.*

No. of goat.	Sex.	No. of cocci inoculated.	Bulk of infective milk.	Method of inoculation.	Serum reaction.		Duration of experiment in days.	*Post-mortem* findings.					
					Day of appearance.	Amount of dilution.		Value of serum.	*M. melitensis* recovered from—				
									10 c. mm. of blood.	Inguinal glands. R.	Inguinal glands. L.	Mesenteric glands.	Spleen.
25	♀	5,600	c.c. 0·2	Infective milk rubbed into prepared area of skin.	15	1 : 20	20	1 : 100	–	+	+	–	+
23	♀	11,200	0·4		—	—	18	—	–	–	–	–	–
Cf. also Monkey No. 188	♂	13,250	0·2		13	1 : 50	17	1 : 200	+	+	+	+	+

Goat No. 117 was then deposited on the centre of the prepared area and rubbed into the skin with the top of the forefinger—still protected by the rubber gloves. When dry, the kid was released, isolated in a separate stall, and subsequently specimens of blood were examined from time to time with negative results until the eighteenth day after inoculation, when the kid, which had been refusing its food for nearly a week, died.

According to the animal attendants, death was due to the fact that an inferior batch of fodder, on which it had been feeding, was an unsuitable food for so young a goat. Be this as it may, at the *post-mortem* examination, beyond absence of fat and emaciation, no obvious cause for death could be detected. *M. melitensis* could not be found in any of the organs nor in the subcutaneous tissue or skin at the seat of inoculation, and specific agglutinins were absent from the blood serum.

The successful result attending the cutaneous method of inoculation in the case of Goat No. 25/6—a method closely resembling that employed in Jennerian vaccination—renders it extremely probable that convection by the flies which positively swarm in the goat pens, may play a part in the transmission of the infection from goat to goat. First settling on drops of infective milk or urine, soiling body and legs, as well as proboscis, and then flying to a scratch or friction ulcer on a previously healthy goat, whether on udder or other part of the body, it would be a very easy matter for the fly to deposit a sufficient number of *M. melitensis* on the raw surface to ensure infection.

The result of the foregoing experiments, together with some observations made in connection with inoculation through the apparently uninjured mucous membrane of the external genitals of the monkey (*vide* p. 113), render the probability of infection of the goat during impregnation extremely likely—a probability which, so far as the male is concerned, is rendered still more likely owing to the fact that the female urethra opens into the floor of the vagina a couple of centimetres or more within the orifice of the canal, so that when the animal is passing *M. melitensis* in the urinary excretion the coccus must be present near the mouth of the vagina in considerable numbers. Unfortunately it was not feasible to test this method of infection experimentally, for, although numerous healthy and infected males were forthcoming, the late season rendered efforts to secure healthy and infected females in suitable condition for such experiment fruitless.

Transmission of the coccus by means of blood-sucking flies, other than by mechanical conveyance upon body or limbs, for example, by *Stomoxys calcitrans*—which abounds in the goat stalls—appeared from the experiments carried out during the summer to be extremely improbable. The negative results obtained in this connection are detailed later (see p. 99).

6. *The Localisation of* M. melitensis *in the Infected Goat.*

Inoculation of the goat with *M. melitensis* produces what must be regarded as an acute, or more commonly a sub-acute, septicæmia—the specific organism living and multiplying in the circulating blood for a variable, often a considerable period—which but rarely proceeds to a fatal termination.

Except on rare occasions when for the few days immediately following inoculation the temperature is raised, the constitutional disturbance accompanying infection is but little marked, and soon ceases to be demonstrable; later on the presence of the specific organism can be detected in excretions and secretions, viz., the urine or milk, or both; but these phenomena are by no means constant. Observations were therefore undertaken as opportunity offered with a view to tracing the route followed by *M. melitensis* in the course of the disease as observed in goats, by studying the *post-mortem* localisation in cases of different durations in the light of the information derived from similar observations on the course of the disease in the monkey.

The results obtained, however, are extremely conflicting (*vide* Table X), and the general statement to be formulated immediately, although probably true in the great majority of goat infections, can only be accepted at the present stage of the investigations as a pious opinion or a working hypothesis.

During the early stages of the infection the blood stream and the spleen are the chief seats of *M. melitensis* activity: later the coccus becomes localised to the speen, lymphatic and other glandular structures and kidneys, probably by the operation of a process akin to filtration, but during its existence in the spleen the possibility of its re-appearance in the blood is ever present. Later still those foci present in the kidneys are destroyed, next those in the spleen, then those in the general lymphatic glandular system, so that in infections of long duration the coccus may be absent from all and every organ with the exception of the mammary gland, where it may persist for very extended periods.

In addition to tabulating the observations upon which the opinions enunciated above are based, it will be well to discuss in order the chief organs and tissues from which *M. melitensis* has been isolated.

1. *The Blood.*—Zammit, working with naturally infected goats, noted that "in certain phases of the disease the specific microbe circulated freely in the blood, but that this condition did not last long."* Again, Eyre, whilst using living cultures in the attempt to immunise the goat and the horse, found that living cocci could be isolated from the peripheral blood for about four weeks after an injection.†

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* *Vide* these Reports, IV, p. 97.

† Reports, V, pp. 45 and 49.

During the period of incubation, that is to say, between the moment of actual infection and the rapid multiplication of the coccus in the circulating blood, usually some 7 to 21 days, attempts to isolate the coccus from peripheral blood are invariably unsuccessful.

Referring once more to the immunising experiments, it was observed that during the first fortnight after the intravenous injection of living cultures of *M. melitensis* it was a comparatively easy matter to isolate the coccus from peripheral blood, although in gradually decreasing numbers, by simple cultivation methods; by the third week the numbers present were small, and after the fourth week it was not possible to detect the presence of the micrococcus either by cultivation or inoculation experiments.

Then, too, in none of the animals tabulated below was the specific coccus isolated from the heart blood, although one was examined *post mortem* three weeks after infection.

Zammit, however, remarked that some of the goats from whose blood he isolated the micrococcus were undoubtedly cases of long standing, a statement based on a consideration of the physical condition of the goats to which he refers; and the fact that two of them were in poor condition and were passing *M. melitensis* in the milk, lends support to the inference. While it is true that these cases might really have been recent infections, the physical condition and presence of the coccus in the milk being due to the exceptional severity of the infection, this is hardly likely; and the more probable explanation is that in cases of long duration, temporary re-infection of the blood stream sometimes occurs from foci in the spleen—a condition analagous to that observed in man during the course of protracted cases of Malta Fever.

In the majority of cases, therefore, the ready isolation of the coccus from the peripheral blood by cultivation methods would afford strong support to a diagnosis of recent infection.

Spleen.—As will be seen from the synopsis of *post-mortem* findings in Table X, it is quite the rule to isolate the micrococcus from the spleen in cases of short duration and up to about 12 months after infection, at any rate where the infection has been experimentally produced. In animals where the infection has existed for a considerable period (many of the *post-mortems* being carried out on animals which were naturally infected when purchased and had been under observation for periods extending over 12 to 20 months) it is the exception rather than the rule.

Lymphatic Glandular System.—There is as a rule in cases of long duration, as well as the more recent ones, a general enlargement of the lymphatic glands, from all of which the *M. melitensis* can be isolated. In recent infections those glands draining the location of the primary infection contain numerically more of the cocci than those which have

Table X.—Results of *Post-mortem* Examinations of Goats.

No.	Sex.	Remarks.	Duration of infection or period of observation.	Presence of *M. melitensis* noted during life.		Serum value at death.	*Post-mortem* findings. *M. melitensis* present in—					
										Glands.		
				Urine.	Milk.		Heart blood.	Spleen.	Kidney.	Inguinal.	Mesenteric.	Udder.
			weeks.									
25	♀	Cutaneous inoculation ...	3	—		1 : 100	—	+	—	+	—	0
10	♂	Subcutaneous inoculation	6	—	—	1 : 200	—	+	—	+	—	0
9	♂	” ”	6	—	—	1 : 100	—	+	—	+	+	0
5	♀	” ”	6			1 : 20	—	+	—	—	—	0
4	♀	” ”	6	—	—	1 : 20	—	+	—	—	—	0
Rosso	♀	” ”	21	—	—	1 : 1500	—	+	—	—	+	0
XXV	♀	Fed with infected milk...	26	—	—	1 : 100	—	+	—	+	—	0
IV	♀	Fed with emulsion of culture	37	—	At 7 wks.	1 : 20	—	—	—	—	—	—
Bianca	♀	Subcutaneous inoculation	39	—	—	1 : 20	—	+	—	—	—	0
VIII	♀	Fed with infected milk...	41	—	—	1 : 10	—	—	—	—	—	0
XII	♀	Fed with emulsion of culture	42	—	—	?	—	+	—	—	—	0
XIII	♀	Fed with urine infected dust	45	—	At 13 wks.	1 : 10	—	—	—	—	—	0
X	♀	Fed with infected milk...	49	—	At 20 wks.	1 : 20	—	—	—	—	—	+
XI	♀	?	49	—	—	1 : 50	—	—	—	—	—	—

VII	♀	Fed with emulsion of culture	53	—	At 43 wks. to day of death	1 : 160	—	—	—	—	—	0
102	♀	Naturally infected.........	19	—	—	1 : 20	—	—	—	—	+	0
103	♀	,, ,, ...	19	—	—	1 : 20	—	—	—	—	—	0
118	♀	,, ,, ...	19	—	—	1 : 10	—	—	—	+	—	0
XIX	♀	,, ,, ...	43	—	At 20 wks.	1 : 160	—	—	—	—	+	+
III	♀	,, ,, ...	56	+	+	?	—	—	—	—	—	0
I	♀	,, ,, ...	60	+	+	?	—	+	+	—	—	0
II	♀	,, ,, ...	60	+	+	?	—	—	—	—	—	0
V	♀	,, ,, ...	60	+	+	?	—	—	+	—	—	0
VI	♀	,, ,, ...	60	+	+	?	—	—	—	+	—	0
XV	♀	,, ,, ...	60	—	—	?	—	—	+	—	—	0
XVI	♀	,, ,, ...	60	—	—	?	—	—	—	+	+	0
XVII	♀	,, ,, ...	60	—	—	?	—	—	—	+	—	0
XVIII	♀	,, ,, ...	60	—	—	?	—	—	—	—	—	0

— = *M. melitensis* not recovered. + = *M. melitensis* recovered. 0 = not examined.

merely derived their bacterial content from the circulating blood, and in the very early stages (*vide* Goat No. 25) the former glands may alone contain the coccus in demonstrable quantities.

Towards the termination of the infection the lymphatic glands—particularly the inguinal—appear to be the last resting place of the coccus before its final destruction, and in a previous report* this fact has been commented upon by various workers who have expressed the opinion that had the examination of the superficial lymphatic glands in the case of many infections of long standing been neglected, *positive* evidence of infection by the isolation and identification of the *M. melitensis* would have been entirely missed. On the other hand, the evidence available by no means justifies the opinion, also there expressed, that the lymphatic glands were the site of especial reproductive activity on the part of *M. melitensis.* That the glandular infection is not limited to those glands draining the area first infected is well shown in the *post-mortem* results of inoculated monkeys (*vide* pp. 34, 51, and 114) which were examined in the early stages of the disease. In the cases, for instance, of food infection, inguinal and axillary glands as frequently yielded a copious growth of *M. melitensis* as the mesenteric (or bronchial and cervical, although these last are not included in the tables); and in the case of a cutaneous infection (Monkey No. 188) as well as in those of mucous membrane infection (Monkeys No. 200 and 203) when the mesenteric glands gave a growth equal to that from any of the subcutaneous glands.

Kidney.—Although the presence of *M. melitensis* in the tissues of the kidney practically synchronises with the appearance of numerous cocci in the blood stream, the extrusion of the micro-organism in the urine is in the majority of the experimental animals a somewhat late phenomenon. In man, on the other hand, the coccus may be demonstrable in the urine quite early in the acute stages of an attack of Malta Fever. In the rabbit and guinea pig its appearance—although usually late—is fairly constant; in the goat, however, the elimination of the coccus is by no means constant even in cases of very long standing, and it is of interest to record the fact that frequently repeated examinations were made of the urine from all of the infected milch goats, some 20 in number, under observation at the Lazzaretto over a period of about a couple of months without the presence of the coccus ever being detected—although 18 of the animals were eliminating large numbers of the coccus almost daily in the milk. Again, the discharge of the coccus in the urine in cases where it has been observed appears to cease long before its disappearance from the milk.

Mammary Gland.—In the few *post-mortem* observations that were made as to the presence of *M. melitensis* in this situation, it may be noted that the organism was detected distributed throughout the substance of the gland in the case of a goat that had previously yielded the coccus in its milk but had been "dry" for some weeks before it was killed. In three other cases small pieces of the base of the udder, containing the deepest portions of the glandular tissue, were removed. In one (where the coccus had appeared in the milk after the lapse of seven weeks from the date of infection) killed eight months after infection, the coccus could not be recovered from the mammary gland or any other of the organs examined. In another which had never shown *M. melitensis* in the milk during life, and which was killed ten months after experimental infection, the micrococcus was absent from all organs and tissues. In the third, killed 12 months after experimental infection, the coccus was isolated from the mammary gland alone of all the tissues examined.

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* *Vide* these Reports, IV, p. 69.

Where the milk has been systematically examined in animals that have been experimentally infected, the appearance of the coccus in the milk has invariably been a late phenomenon. Goat No. 4, for example, commenced to eliminate the coccus seven weeks after infection, Goat No. 8 three months. Goat No. 7, on the other hand, was infected in September, 1904, and, although repeatedly examined, showed no signs of the coccus in the milk up to January, 1906, when, being "dry," she was impregnated. In July, 1906, she dropped two kids, and three days later the coccus appeared in the milk and was consistently present up to 24th September, when she was slaughtered, and it is interesting to note that in this case the coccus could not be recovered *post mortem* from either spleen, kidneys, or lymphatic glands.

7. *The Transmission of* M. melitensis *Anti-bodies to the Descendants of Infected Goats.*

A further interesting point, and one which has a considerable bearing upon practical preventive measures against the spread of *M. melitensis* infection in the goat, is the fact that while an appreciable amount of specific agglutinin is usually transferred from the infected milch goat to its offspring, the *M. melitensis* itself, minute though it is, does not appear to cross the placenta.* These observations were repeated on several occasions as opportunity offered, and the details concerning four kids born of experimentally-infected mothers (all of which were passing *M. melitensis* in either milk or urine, or both, up to the time of impregnation, and even after), may be tabulated as follows:—

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* One observation upon the human subject where transference of the micrococcus from the maternal to the fœtal circulation appears to have taken place *in utero* may here be referred to. A pregnant woman (under the care of Captain Williams, R.A.M.C., in the Married Families Hospital, Valletta) gave birth to a male infant during one of the pyrexial periods of an acute attack of Mediterranean Fever. The infant proved to be infected also, the agglutination value of its serum was 1 in 1000—as compared with its mother's 1 in 500—and its temperature curve from birth followed precisely that of the mother, although about half a degree lower, during the subsequent fluctuations.

Milch goat.	Kids born.	Serum value in kid.	Kid killed.	*Post-mortem* results *qua M. melitensis.*
1	25.10.05	1 : 50	13.11.05	Nil.
2	2.3.06	1 : 800	4.3.06	Nil.
3	14.7.06	*a* 1 : 50	31.7.06	Nil.
		b 1 : 20	31.7.06	Nil.

Even the small amount of agglutinin present in the serum of the kids of infected goats appeared to be associated with a very definite immunity, for the two kids of Goat No. 3 were fed for 17 days on their mother's milk, and for the last 10 days of this period the milk was teeming with *M. melitensis*, yet, at the *post-mortem* inspection, no trace of *M. melitensis* infection could be detected.

On the other hand, two kids which had been dropped, and subsequently reared by a similarly infected mother, were injected subcutaneously, when they were seven weeks old, with emulsion of cultivation of *M. melitensis*, and were quite unable to resist infection, showing that the resistance offered to invasion by *M. melitensis* can readily be overcome.

8. *The Treatment of Infected Milch Goats with the* M. melitensis *Vaccine.*

The lengthy periods over which apparently healthy milch goats continue to secrete milk of a highly infective character rendered it desirable to inquire into the utility of therapeutic measures in determining the duration of active infection. To this end observations were commenced with regard to the effect of treatment by *M. melitensis* vaccine, as it appeared useless to try any of those drugs that had already proved useless in the treatment of *M. melitensis* infection in the human subject.

Consequently, early in August a batch of vaccine was prepared from a highly virulent strain of *M. melitensis* in the manner described in Section VII, p. 116, and standardised to 1000 million micrococci per cubic centimetre. Seven of the herd of infected milch goats and sheep stabled in the Lazzaretto were selected for the purpose of the experiment—four for treatment, Nos. 104, 111, 114, and 117; and three—105, 107 (sheep), 115—to serve as controls; the milk from all of these animals usually contained *M. melitensis*—often in enormous numbers—as will be seen by reference to Table VII, p. 20. Unfortunately, Goats Nos. 105 and 111 "dried up" shortly after the experiment was started.

The bi- or tri-weekly enumeration of the cocci present in the milk of these seven animals was continued, and observations on the agglutination reaction in serum and milk were also carried out.

Table XI.—*M. melitensis* in Milk of Vaccinated Goats and Controls.

Animal and No.	Goat 104.	Goat 114.	Goat 117.	Controls.	
				Goat 115.	Sheep 107.
Aug. 2 ...	16,000	30,000	24,800	—	1,810
„ 4 ...	—	30,000	—	730	—
„ 7 ...	660	—	—	—	1,000
„ 8 ...	Injected with vaccine (500,000,000 cocci)				
„ 9 ...	1,250	8,300	1,200	3,800	300
„ 11 ...	50	3,000	16,000	3,500	2.500
„ 14 ...	1,600	nil	30,000	8,000	20,000
„ 17 ...	1,500	—	3,400	—	4,200
„ 18 ...	750	6,000	—	—	720
„ 21 ...	1,400	200	2,000	—	1,500
„ 21 ...	Injected with vaccine (1,000,000,000 cocci)			—	—
„ 23 ...	700	5,000	200	1,020	20,000
„ 25 ...	2,000	—	30,000	3,200	30,000
Sept. 4 ...	—	38	525	nil	700
„ 6 ...	110	—	1,161	360	1,600
„ 8 ...	176	—	471	60	2,280
„ 11 ...	127	300	200	560	2,245
„ 15 ...	139	800	*	1,200	3,456
„ 15 ...	Injected with vaccine (1,000,000,000 cocci)			—	—
„ 18 ...	185	*	207	350	4,300
„ 20 ...	*	nil	320	560	5,280
„ 22 ...	nil	*	170	150	*
„ 25 ...	10	20	360	100	*
„ 27 ...	65	15	230	50	5,300

* Plates so contaminated as to be unworkable.

The initial dose of vaccine was 0·5 c.c., equivalent to 500,000,000 cocci, which was injected subcutaneously in the animal's flank. In the course of 12 hours or so the small local swelling caused by the injection had completely disappeared, the animal seemed to be in no way discommoded by the injection, and no marked rise of temperature took place. Within two or three days the agglutination value of both serum and milk fell in a most pronounced fashion, and on one occasion, in the case of Goat No. 114, disappeared altogether from the milk. Four or five days later the dilution in which the agglutination reaction could be obtained had again risen to a higher level than it had occupied previous to the injection of the vaccine—in short, the phenomena observed were exactly comparable to those noted in rabbits and guinea-pigs under similar conditions.* The numerical strength of *M. melitensis* in the milk at first appeared to be as irregular and as erratic as before the treatment was commenced, but after a second injection of a somewhat larger dose of vaccine (1 c.c., or 1,000,000,000 cocci) 14 days after the first, the numbers underwent a certain diminution. Unfortunately, about this time the milk of another of the controls commenced to dry up, and within a month of the commencement of the experiment only small quantities of milky fluid could be obtained from this animal for examination. The Sheep No. 107, however, continued to yield a good supply of milk containing *M. melitensis* in undiminished numbers. These observations are being continued, for it is obvious that any method of treatment which would cause the destruction of the micrococcus in the infected goats and its disappearance from the milk would be a most valuable weapon in the hands of those who desire to stamp out Malta Fever from our Fleet and garrisons.

In the accompanying table (p. 43), which gives the number of cocci per cubic centimetre in the milk of the treated goats and in the controls, the diminution in the former may be readily followed.

II.—*M. melitensis* INFECTION BY EXPERIMENTAL FEEDING WITH INFECTIVE GOATS' MILK.

1. *General Considerations.*

The experimental work undertaken by the Commission during the years 1904 and 1905 proved beyond question the possibility of producing a generalised infection in monkeys, goats, and kids as the result of feeding them upon articles of diet artificially contaminated with *M. melitensis*, although many of the earlier experiments were negative, and the value of some of the later positive experiments is depreciated owing to their lengthy duration, which affords opportunity for the introduction of numerous fallacies. For convenience of reference and comparison, these early experiments in the monkey dealing with infection *viâ* the alimentary system, have been tabulated (see pp. 46 and 47).

The method of experimentation that was adopted (*i.e.*, the repeated administration of contaminated food on successive or alternate days, often for long periods of time), combined with the difficulty of demonstrating the presence of *M. melitensis* on every occasion that supposedly contaminated articles of diet were administered, left many points of interest for further investigation.

Perhaps the most important of these was the question whether the administration of one quantum of contaminated food was sufficient to produce an *M. melitensis* infection *viâ* the alimentary tract, for, if so, the equally important question of the duration of the incubation period of the disease would necessarily be further elucidated.

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* *Vide* these Reports, V, p. 44.

From the experiments tabulated below, it would at first sight be assumed that enormous doses of the virus are required to produce infection, and that the period of incubation, though varying within very wide limits—from 14 to 76 days—was usually a lengthy one; but a careful consideration of the various factors involved in those experiments is sufficient to show that such an assumption might well be fallacious, for the simple reason that the appearance of specific agglutinins in the blood serum was the criterion adopted as the evidence of infection. Now, it has already been shown elsewhere in these Reports (*vide* Part V, p. 45) that an animal inoculated with an excessively large dose of the micro-organism, or repeatedly injected with dead or with living cultures of *M. melitensis,* either fails altogether to elaborate specific agglutinins, or if such are present at the commencement of the experiment, fails to elaborate fresh supplies, whilst that previously stored in the serum is rapidly destroyed. An obvious explanation of the very lengthy incubation periods would therefore be that many of the animals to which repeated doses of *M. melitensis* were administered in the shape of daily supplies of food infected with the micro-organism, failed to form the specific agglutinins either until very late in the course of the infection, or until the temporary cessation of the "feeding," or the accidental absence of *M. melitensis* from the presumably infected food afforded the necessary respite, and allowed the response to the presence of the micro-organism to emerge from the negative into the positive phase. Again, the experimental inoculation of laboratory animals (*e.g.*, guinea-pigs, and particularly white rats) has shown that death frequently takes place when even a 1 in 10 agglutination reaction is absent and has been absent throughout the course of the infection, while the *post-mortem* examination reveals the fact that all the organs are teeming with the *M. melitensis.*

In support of this view, the case of Monkey No. 2 may be quoted (see Table XII). This animal was—with the exception of one day—fed continuously for 74 days with infective milk, and during this period no agglutination reaction could be obtained with the blood serum. The day following the cessation of the feeding, a very doubtful 1 in 10 reaction was obtained; nine days later the animal suddenly died, and at the *post-mortem* examination *M. melitensis* was recovered from spleen and from glands, although the blood serum still yielded merely an incomplete reaction with a 1 in 10 dilution.

These facts make it a matter of regret that the supply of animals for the early experiments was insufficient to allow of any considerable number being utilised for each experiment. Had it been possible to include more animals in certain of the experiments, so that some might have been killed and examined *post-mortem* during the course of the feeding, it would probably have been found quite frequently that infection had taken place at a very much earlier stage than was indicated by the appearance of the serum reaction.

Table XII.—Feeding Experiments 1904 and 1905.—Monkeys.

No. of experimental monkey.	Duration of "feeding" in days.	No. of "feeds."	Dose of *M. melitensis* at each feed.	Method of administration.	Evidence of infection.			Observer.	Reference to volume and page of these Reports.
					Presumptive.		Absolute.		
					Appearance of serum reaction at	Value of serum.	*M. melitensis* recovered from—		
					days.				
39	32	28	1 agar slope	Culture from human spleen mashed with boiled potato	31	1 : 500	Spleen	H.	I, 50
40	33	28	"		32	1 : 10	Nil	H.	I, 53
113	13	12	?	Naturally infected urine (human) mixed with dust and dried, then sprinkled on food	—	—	Nil	H.	IV, 30
114	55	55	?		—	—	Nil	H. & K.	IV, 31
119	29	22	?		28	1 : 100	All organs	H.	IV, 35
120	29	29	?	Naturally infected urine (human) spread on potato	—	—	Nil	S.	V, 15
121	30	30	?		—	—	*		
26	134	39	?		—	—	*		
122	203	76	?	Naturally infected urine (human) mixed with dust and dried, then mashed up with potato	58	1 : 30	Kidney	S.	V, 16
30	69	63	?		—	—	Nil		
123	80	43	?	... ...	75	1 : 30	All organs		
124	29	29	1 agar slope	Culture from human spleen smeared on potato	13	1 : 30	Superficial glands only	S.	V, 17
125	29	29	"		28	1 : 40	Femoral and axillary glands		

126	29	29	,,	Culture from human urine smeared on potato	17	1 : 80	Nil	S.	V, 18
127	29	29	,,		28	1 : 120	Superficial and mesenteric glands		
2	74	73	?	Mixed milk from infected herd of 11 goats	74 ?	1 : 10	Spleen and glands ...	H. & K.	IV, 58
4	32	78	?		31	1 : 40	,, ...	H. & K.	IV, 56
5	30	70	?		29	1 : 10	,, ...	H. & K.	IV, 54
99	65	31	?	... Variously with mixed milk from infected herd and whole milk from one of three infected goats	65	1 : 10	Mesenteric glands ...	H. & K.	IV, 59
6	24	8	1 agar slope	Culture from goat's milk mashed up with potato	31	1 : 10	All organs.............	H. & K.	IV, 48
7	24	9	,,		35	1 : 50	Spleen and glands ...	H. & K.	IV, 49
8	24	7	,,		25	1 : 10	Axillary, femoral and mesenteric glands	H. & K.	IV, 50
9	24	9	,,		25	1 : 10	Femoral and axillary glands	H. & K.	IV, 51
19A	2	2	,,	... Culture from goat's milk emulsified	33	1 : 10	Spleen and glands ...	H. & K.	IV, 52

* No *post mortem* performed. H. = Horrocks. K. = Kennedy. S. = Shaw.

Basing a working hypothesis upon these considerations, and planning the experiments so that the results might yield the maximum of information, a considerable number of feeding experiments were performed, which for convenience of study are divided into six series. The main object of the experiments was to obtain exact information of the effects, if any, produced by the ingestion of a limited quantity of infective material; therefore it was determined to administer to each animal but one quantum of infected food, then, after a few days' interval, to commence testing the blood for the presence of agglutinins, and thenceforth to continue to apply this test on alternate days; and from the third week onwards to kill and examine *post-mortem* one or more animals each week. These experiments may now be described as follows :—

2. *Feeding with Naturally Infected Milk Artificially Reinforced.*

Series I.—Eight monkeys (*Macacus rhesus*) were employed in this experiment.

From the time of their arrival from Calcutta these animals were kept under observation at the Lazzaretto as to temperature and general condition, and were fed on soft food—boiled rice and boiled potato, together with sugar and water, and the blood serum from each repeatedly examined for *M. melitensis* agglutinins. The general condition being satisfactory, no lesions of the mucous membrane of the mouth, or of the skin of the face, hands, or feet being apparent to visual inspection, and specific agglutinins being absent from the blood, the monkeys were brought over to the laboratory at Valletta and arranged on the roof terraces. Each monkey was fastened in close proximity to a separate wooden cage by means of a chain, rather more than a metre in length, fastened at one end to a staple in the wall, and at the other to a ring in a leathern dog-collar secured around the animal's neck. Each monkey was separated from his neighbour on either side by a wooden partition, some 6 feet in height, projecting at right angles from the wall on which the cage was fastened for about 6 feet, and set in cement at its junction with both wall and pavement. By this means not only was personal contact between a monkey and its neighbour or neighbours rendered impossible, but the chance of contact with food or excrement other than its own was also obviated. In addition to the soft food previously mentioned, each monkey was supplied with about 250 c.c. of sterilised goats' milk per diem for three days. This they readily learned to lap up as they did water. On the fourth day they had the usual morning feed, together with sterilised milk at 8 a.m., but no more food was given that day. The following morning, May 13, 1906, the administration of infected food—goats' milk—took place. The infected milk employed in this experiment was "mixed milk" derived from a herd of nine goats just received at the Lazzaretto from Rabato, five of which were known to be excreting *M. melitensis* in varying numbers in their milk, while the remainder were suspected of doing so on account of the agglutination reaction yielded by the milk, and the fact that *M. melitensis* had been isolated from the milk before they passed into the Commission's possession, although the micro-organism had not been recovered from the milk during their stay in the Lazzaretto up to the date of the experiment.

Having regard to the fact that any given milch goat shows great variations in the number of *M. melitensis* excreted in the milk even from day to day, a single agar slope culture of *M. melitensis* derived from the milk of Goat No. 104 was emulsified in sterile saline solution, and added to the two gallons of milk obtained from the infected herd. Plate cultivations were then prepared from the milk itself, and from various dilutions thereof, and after incubation at 37° C. for four days the colonies of *M. melitensis* which had developed were enumerated, and it was finally estimated that each cubic centimetre of the infected milk when supplied to the experimental monkeys contained 11,000,000,000 *M. melitensis.* That is to say, on this occasion, the milk contained *M. melitensis* to the number of about 10,000,000,000 per cubic centimetre *before the laboratory culture was added.*

The method of administration of the infective material was quite simple. As the monkeys were accustomed to receive sterilised milk as a regular article of the daily dietary, a small amount, 250 c.c., was supplied to each monkey in a clean pannikin at 9 a.m. From the laboratory window the animals were watched, and it was seen that each lapped up a certain quantity of the milk, in most cases small in amount, or, lifting up the pannikin, drank from it as one would from a cup before turning the receptacle upside down to ascertain whether other more solid food was concealed beneath. At 11.30 a.m. the pannikins were cleaned and a further 250 c.c. supplied to each animal, when the same performance was gone through. Each animal, in all probability, consumed 30 c.c. to 50 c.c. of the milk; certainly no animal ingested as much as 100 c.c. At 2 p.m. the usual feed of boiled rice was given, and from that time forward the ordinary meals were supplied and no more infected material was administered.

To serve as controls two healthy monkeys were each supplied with sterilised goats' milk in similar quantities, and as the two following series were carried out on the same day, they acted as controls for those also.

Six days after the administration of the infected milk a specimen of blood was taken from each monkey and tested for the presence of *M. melitensis* agglutinins. The result in each case was negative. This testing for the serum reaction was repeated thenceforth three or four times every week until the termination of the experiment. On the 10th day after feeding the first agglutination reaction was observed

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(1 : 10); on the 12th day three other monkeys gave a good serum reaction in low dilutions (1 : 10, 1 : 20, and 1 : 20), while the first animal gave only an incomplete reaction in 1 : 10. On the 15th day three more monkeys reacted 1 : 10, 1 : 10, and 1 : 80 respectively, and on the 20th day the eighth monkey of the series yielded a 1 : 10 reaction. On this day also Monkeys Nos. 160 and 161 were chloroformed and autopsies performed, and in these, as in all *post-mortems* conducted in the laboratory, a thorough examination was carried out. In addition to the usual naked eye inspection, plate and tube cultivations were invariably established from the spleen, the axillary and inguinal glands, and at least four mesenteric glands; 10 cubic millimetres of blood taken directly from the heart were plated out, and a further supply of blood was collected for the purpose of determining the agglutinating value of the serum at the time of death.

Naked eye inspection of Monkeys Nos. 160 and 161 showed nothing beyond general glandular enlargement, and hypertrophy of the spleen, which was of a dark colour, hard and friable; but as the result of the bacterioscopic examination both these animals were found to harbour *M. melitensis* in the blood and in every organ examined.

Eight days later, that is four weeks from the day of feeding, one of the control monkeys was killed by chloroform and examined, but the result of the autopsy was completely negative. All the organs appeared normal, the blood serum possessed no agglutinating power whatever when tested against *M. melitensis;* nor could *M. melitensis* be demonstrated in any of the organs or tissues examined culturally. The second control was killed on the 36th day, and yielded identical results. Two more animals of this series were killed and autopsies performed on the 32nd day after feeding, the *M. melitensis* being recovered from one or more organs in each case, the spleen invariably yielding a growth of the micro-organism.

The full details of this experiment are tabulated below, while the net result may be summarised by saying that of eight experimental monkeys fed once on somewhat grossly infected milk, eight became infected by *M. melitensis* (as proved by results of *post-mortem* examination) after an incubation period varying from 10 to 20 days (as indicated by the date of appearance of the serum reaction).

Series II.—To contrast with the first series of animals, a further batch of similarly selected monkeys (from the same Indian consignment) were simultaneously prepared for feeding. In substitution for the rice and potatoes in the diet supplied to the animals in Series I, the monkeys in Series II received for the corresponding three days medlars, small Spanish nuts which they cracked between their teeth in order to get at the kernels, and hard roasted peas, the object being to afford the animals every opportunity of abrading the mucous membrane of the buccal cavity, and so facilitating the entrance of

Table XIII.—Feeding Experiments in 1906.—Series I.

No. of monkey.	Sex.	Preparation for feeding.	Dose of infective material.	Method of administration.	Serum reaction.		Duration of experiment in days.	*Post-mortem* findings.						
									M. melitensis recovered from—					
					Day of appearance.	Amount of dilution.		Value of serum.	10 c.mm. of blood.	Axillary glands. R.	Axillary glands. L.	Inguinal glands. R.	Inguinal glands. L.	Mesenteric glands.	Spleen.
162	♀	Two meals daily, comprising boiled potatoes, boiled rice, and sterilised milk, May 9, 10, and 11. One similar meal at 8 A.M., May 12.	Not more than 50 c.c. infected goat's milk, containing 11,000,000,000 *M. melitensis* per c.c (Naturally infected goat's milk, reinforced by addition of 1 agar slope culture *M. melitensis*, derived from goat's milk, to 2 gals. of milk.)	Each monkey supplied with 250 c.c. of milk, in an open pannikin, at 9 A.M., and a further 250 c.c. of same milk at 11.30 A.M., May 13, 1906.	10	1 : 10	36	1 : 500	–	–	–	+	+	–	+
159	♂				12	1 : 10	36	1 : 200	–	–	–	–	–	–	+
160	♂				12	1 : 20	20	1 : 100	+	+	+	0	+	+	+
161	♂				12	1 : 20	20	1 : 50	+	+	+	+	+	+	+
163	♂				15	1 : 10	36	1 : 50	–	–	–	–	–	–	+
164	♂				15	1 : 20	36	1 : 200	+	+	+	+	+	+	+
165	♀				15	1 : 10	32	1 : 80	+	+	+	+	+	+	+
23	♀				20	1 : 10	32	1 : 600	+	+	+	+	+	+	+
Controls. 150	♂		About 50 c.c. sterilised goat's milk		—	—	36	—	–	–		–		–	–
172	♀				—	—	28	—	–	–		–		–	–

M. melitensis—converting, in fact, the feeding experiment into a subcutaneous inoculation, and imitating that condition of the mouth which must be frequently present in human subjects resident within the endemic area of Mediterranean Fever—in the anticipation that earlier infection associated with severer constitutional symptoms would sufficiently indicate that the desired end had been attained. Anxiety to ensure the ingestion of the infected food appears, however, to have caused the failure of this portion of the experiment, for the monkeys in Series II received their last lot of nuts and peas on the morning of May 12, and, like the animals in Series I, received no more food until the infected milk was placed before them 24 hours later, by which time any small abrasion caused by cracking nuts would, most probably, have been sealed off from contact with the contents of the mouth by a protective coating of serum.

Of this batch the first monkey gave a serum reaction of 1 : 10 on the 12th day after feeding with infected milk, four reacted for the first time on the 15th day and two more on the 20th day. One cubic centimetre of blood was abstracted from the external saphenous vein (by means of a small serum syringe) of Monkey No. 157 on the 18th day after feeding, and planted into 20 c.c. of broth. After three days' incubation at 37° C. of this first broth reinforcement, plate cultures therefrom yielded a pure culture of *M. melitensis.* Six of the remaining monkeys were killed on the 21st, 32nd, 36th, and 37th days after feeding, and cultural examination of the various organs specified in connection with Series I yielded abundant evidence of generalised infection by *M. melitensis.*

The eighth animal of Series II alone remains to be accounted for. This monkey gave a not quite complete reaction in a dilution of 1 : 10 on the 10th day after feeding; on the 35th day a similar reaction in 1 : 40 dilution.

It was killed by chloroform vapour on June 25, when it gave similar, not absolutely complete, reactions in all dilutions up to 1 : 40. At the autopsy general glandular enlargement of the axillary and inguinal glands was noted, associated with visible distention of the superficial lymphatics. The mesenteric glands were enlarged—some purulent, others caseous, and coverslip film preparations from both varieties, stained by the Ziehl-Neelsen method, showed the presence of acid-fast bacilli morphologically indistinguishable from *B. tuberculosis.* The spleen was large, dark, and friable, and studded all over with miliary tubercles; the lungs, liver, and kidneys appeared normal—the whole making a typical picture of early generalised tuberculosis. Cultivations from heart's blood, spleen, and the usual glands remained sterile up to the end of 10 days, when the period of observation of the cultivations was brought to a close. The most interesting point in this case was, of course, the pseudo-reaction that was obtained with

Table XIV.—Feeding Experiments in 1906.—Series II.

No. of monkey.	Sex.	Preparation for feeding.	Dose of infective material.	Method of administration.	Serum reaction.		Duration of experiment in days.	*Post-mortem* findings.						
									M. melitensis recovered from—					
					Day of appearance.	Amount of dilution.		Value of serum.	10 c.mm. of blood.	Axillary glands. R.	Axillary glands. L.	Inguinal glands. R.	Inguinal glands. L.	Mesenteric glands.	Spleen.
151	♀	Two meals daily, May 9, 10 and 11, consisting of peas, medlars, Spanish nuts, and sterilised milk. One similar meal at 8 A.M., May 12.	Not more than 50 c.c. naturally infected "mixed" goat's milk reinforced by addition of 1 agar slope culture of *M. melitensis* (derived from goat's milk) to 2 gallons of milk.	Each monkey supplied with 250 c.c. milk in an open pannikin at 9 A.M., and a further 250 c.c. of same milk at 11.30 A.M., May 13.	12	1 : 10	32	1 : 400	+	+	+	+	+	+	+
153	♂				15	1 : 40	37	1 : 4000	+	+	+	+	+	+	+
155	♂				15	1 : 10	36	1 : 1000	—	+	+	+	+	+	+
157	♂				15	1 : 40	*								
158	♂				15	1 : 10	37	1 : 1000	—	+	+	+	+	+	+
152	♂				20	1 : 10	37	1 : 3000	—	+	+	+	+	+	+
154	♂				20	1 : 40	21	1 : 40	—	+	+	+	+	+	+
156	♀				(15)	(1 : 10)	43	(1 : 40)	—	—	—	—	—	—	—
Controls.															
172	♀		About 50 c.c. sterilised goat's milk		—	—	28	—	—	—	—	—	—	—	—
150	♂				—	—	36	—	—	—	—	—	—	—	—

* Still living. *M. melitensis* recovered from peripheral blood on 18th day. Serum reaction on 41st day, 1 : 1000.

the blood serum even up to a dilution of 1 : 40, and at once suggests an explanation of some of the anomalous reactions that are obtained with *M. melitensis* in cases of obscure fever in man.

All the essential details of this experiment (Series II) are tabulated (p. 53), the net result being that of eight experimental animals, one, suffering from *Tabes mesenterica*, was not infected with *M. melitensis*, the remaining seven became infected after an incubation period varying from 12 to 20 days, but presented no especial features as to severity, constitutional symptoms, or of febrile reaction or early appearance of serum reaction that would indicate that infection had taken place by a different path to that traversed in Series I.

This experiment, therefore, although successful in showing the possibility of infection by means of infected food, failed to differentiate between absorption through normal and injured mucous membrane.

Series III.—In a further attempt to investigate the factors involved in the infection through the alimentary system, a third batch of eight monkeys selected for the purpose in a manner similar to that adopted in the two former series were injected subcutaneously, each with 1 c.c. of *B. typhosus* vaccine, a few hours after the morning meal on May 12, with the object of producing a marked constitutional disturbance which should not have passed off by the time the milk infected with *M. melitensis* was administered.

This object was certainly attained—two of the animals in fact succumbed, one being dead on the following morning at 6 a.m., the second dying a few hours after the administration of the infected milk. The remaining six monkeys were clinically distinctly ill, although in no case did the temperature rise above 104° F., repeatedly drank small quantities of the infected milk and refused the rice supplied to them in the afternoon. The following day all were apparently well, and the local swelling marking the seat of injection of the vaccine disappeared completely in the course of a few days.

The results obtained were somewhat conflicting. One animal showed a serum reaction (1 : 10) on the 15th day, and an incomplete reaction (1 : 10) on the 20th day. The agglutinins then disappeared from the blood, and could not again be demonstrated until the 33rd day, when the reaction was obtained in a dilution of 1 : 20. Two more animals reacted on the 20th day, while the sixth, which showed no signs of the presence of agglutinins in the blood, was killed and examined *post-mortem* on the 24th day, after feeding showed absolutely no evidence of *M. melitensis* infection. In two only of the five animals that were infected was *M. melitensis* generalised throughout the body. In the remaining three it was recovered with difficulty—from the spleen alone in two and from the spleen and mesenteric glands in the third.

Despite the danger attending generalisations founded on insufficient premises, it would almost appear that the response of these animals to the injection of *B. typhosus* vaccine had in some obscure manner enabled the majority of them to make a better fight against the invading *M. melitensis*, and to achieve a more rapid destruction of the organism, than any of their fellows who had not been so stimulated prior to the administration of the infected milk. The details of this experiment are given in the accompanying table (p. 56).

As these three series of feeding experiments were carried out simultaneously and under similar conditions of environment, the same two controls served for all, and the details concerning them will be found tabulated with those of the experimental monkeys in each series.

Having by these experiments shown that the ingestion of a small amount of somewhat heavily infected milk was sufficient to determine a *M. melitensis* infection after an incubation period varying from 10 to 20 days, it now remained to ascertain whether naturally infected milk—without reinforcement by the addition of laboratory cultivation —would suffice to yield a like result, and to this end further sets of experiments were initiated. Before detailing these, however, some comment is necessary on the two possible sources of fallacy which may be urged against the foregoing experiments, viz. :—

(A) Infection conveyed by means of mosquitoes and biting flies.

(B) "Place" infection—by reason of the fact that during the last two and a-half years each and all of the wooden cages had been inhabited at various times by infected monkeys, and were, therefore, possibly more or less contaminated with infective excrement.

Dealing first with (A), it should be noted that during April,* May,† and the early part of June,‡ 1906, an exceptionally cold spell of weather prevailed throughout the island, a few Culex (of the common species, fatigans and pipiens) were observed and caught inside houses, but neither they nor Acartomyia or Stegomyia appeared in the Laboratory or on the terrace until long after many of the experimental animals had given unmistakable evidence of the infection. Flies also were conspicuous by their absence; Stomoxys was not observed upon the terraces during these experiments, and it was not until well on in June that it could be obtained, even from stables, in quantities sufficient for experimental work. These points, however, are by themselves insufficient to absolutely negative the objection raised under heading (A).

In the succeeding experiments further precautions were taken for the express purpose of eliminating the possibility of infection through the

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* April, 1906, was the coldest April in the five years 1902—1906, with 26 days in defect of the average for the five-year period.

† May, 1906, was the coldest May in the five years 1902—1906, with 20 days in defect of the average for the five-year period,

‡ June, 1906, was the coldest June in the five years 1902—1906, with 20 days in defect of the average for the five-year period.

Table XV.—Feeding Experiments in 1906.—Series III.

No. of monkey.	Sex.	Preparation for feeding.	Dose of infective material.	Method of administration.	Serum reaction.		Duration of experiment in days.	*Post-mortem* findings.						
					Day of appearance.	Amount of dilution.		Value of serum.	*M. melitensis* recovered from—					
									10 c mm. of blood.	Axillary glands. R.	Axillary glands. L.	Inguinal glands. R.	Inguinal glands. L.	Mesenteric glands.	Spleen.
36	♂	Two meals daily, May 9, 10 and 11, consisting of boiled potatoes, boiled rice and sterilised milk. One similar meal at 8 A.M., May 12.	Not more than 50 c.c. naturally infected "mixed" goat's milk reinforced by the addition of 1 agar slope. Culture of *M. melitensis* (derived from goat's milk) to 2 gallons of milk.	Each monkey supplied with 250 c.c. milk in an open pannikin at 9 A.M., and a further 250 c.c. of same milk at 11.30 A.M., May 13.	10	1 : 20	43	1 : 40	—	—	—	—	—	+	+
171	♀				12	1 : 20	29	1 : 40	+	+	+	+	+	+	+
168	♂				15	1 : 10	43	1 : 20	—	—	—	—	—	—	+
166	♀				20	1 : 10	43	1 : 50	—	—	—	—	—	—	+
170	♂				20	1 : 10	32	1 : 250	+	+	+	+	+	+	+
167	♂				—	—	24	—	—	—	—	—	—	—	—
Controls. 172	♀		About 50 c.c. sterilised milk.		—	—	28	—	—	—	—	—	—	—	—
150	♂				—	—	36	—	—	—	—	—	—	—	—

agency of insects. The animals employed were selected from a consignment of monkeys that arrived in Malta from Calcutta early in July. On their arrival at the Lazzaretto they were placed in mosquito- and fly-proof rooms where they remained until their transference to the Laboratory terraces. Here they were allotted sleeping boxes, each of which was accommodated in a separate cubicle similar to those employed in the first three experiments, but rendered mosquito- and fly-proof by roofing with wood, fronting with a gauze covered framework with hanging door, and caulking all joints and cracks in the woodwork with putty, or pasting them over with stout paper.

With reference to the possibility of infection having taken place through contact with the excrement-polluted woodwork of the sleeping boxes, it may be stated that before allotting a box to a normal monkey, it was first scraped clean, then thoroughly scrubbed inside and out with a 2-per-cent. solution of Lysol, and the wood saturated with the same; then the box was dried in the sun. When thoroughly dry, it was again scrubbed with a fairly strong solution of caustic soda until the wood had regained its pristine whiteness, and no stain or discoloration was visible, well rinsed first with plain water, then with the Lysol solution, and finally exposed to the direct rays of the sun for a day or two.

The cubicle in which the box was to be fitted was thoroughly disinfected by washing down walls and partitions with the Lysol solution (sprayed on by means of a garden squirt), and as the terrace floors are entirely of cement, the same treatment was extended to them.

Finally the two controls and two of the experimental animals failed to contract the disease, and would thus appear to afford proof of the efficacy of the disinfectant measures pursued.

3. *Feeding Experiments with Naturally Infected Milk Alone.*

Series IV.—The same care was observed as in the earlier experiments in picking out animals that were in good general condition, free from cuts and scratches, and from whose blood serum specific agglutinins were absent. The method of preparation for the feeding experiments was identical with that adopted in Series I, and the infective material was again goats' milk derived from the herd of goats under observation at the Lazzaretto. In addition, the monkeys employed in this and the succeeding series had been trained to drink milk from the pannikin "human fashion."

In the fourth set of experiments two monkeys only were employed, and these were each supplied with 500 c.c. of "whole" milk (in two equal quantities as in the previous experiments, the first at 9 A.M., the second at 11 A.M.), the one from Goat No. 114, the other from

Goat No. 115, in order to imitate, as far as possible, the sequence of events taking place in a private household, where the housewife calls to a passing goatherd and receives into the receptacle she provides the milk from one goat for consumption by the family. Each of the goats above-mentioned had been under observation for some months, and was consistently passing *M. melitensis* in its milk. Samples of each of these milks were diluted and plated, and on enumeration after incubation it was found that the milk from Goat No. 114 contained 800 *M. melitensis* per cubic centimetre, and that from Goat No. 115, 300 per cubic centimetre.

The milk was set before the experimental monkeys in pannikins, as in the three former experiments, but, as the animals were shut up in mosquito-proof cubicles, it was difficult to ascertain how much, if any, of the milk had been consumed. With regard to Monkey No. 197, however, it is certain that she ingested at any rate 45 c.c. of the milk from Goat No. 114, as owing to a mistake on the part of an assistant she was anæsthetised during the morning, and a soft rubber catheter passed into her stomach before the error was noticed. Under the circumstances it was deemed advisable to introduce a further dose of the milk she had presumably drunk earlier in the day into her stomach before allowing her to recover from the anæsthetic, and this was accordingly done. Monkey No. 196, on the other hand, only ingested such quantity of milk as she had voluntarily drunk from her pannikin.

Two monkeys which served as "controls" for this and the two following series each received 500 c.c., and consumed about 50 c.c. of sterilised goats' milk.

Of the two animals fed on infected whole milk, Monkey No. 197, which must certainly have received a larger dose of infected milk, and milk moreover which contained at least double the number of cocci per cubic centimetre, showed a definite reaction four days before her fellow sufferer, that is on the 17th day, Monkey No. 196 showing a definite reaction on the 21st day. Exactly one month after feeding, Monkey No. 197 and one of the controls, 189, were chloroformed and careful examinations made of the bodies, with the result that no evidence of infection by *M. melitensis* could be obtained in the case of the control, whilst Monkey No. 197 yielded *M. melitensis* from all the organs examined, although the coccus was absent from the small quantity of heart blood that was plated.

The Monkey No. 196 was chloroformed on the 33rd day, when the blood serum gave an immediate and unmistakable positive reaction in dilutions of 1 in 100. The *post-mortem* examination showed greatly enlarged liver and spleen, each organ being studded with tubercles, many of which were caseous. The superficial lymphatic glands were also enlarged. Cultivations established from the blood and the organs gave rise to a plentiful growth of various bacteria, but no *M. melitensis*

Table XVI.—Feeding Experiments in 1906.—Series IV.

No. of monkey.	Sex.	Preparation for feeding.	Dose of infective material.	Method of administration.	Serum reaction.		Duration of experiment in days.	*Post-mortem* findings.							
									M. melitensis recovered from—						
					Day of appearance.	Amount of dilution.		Value of serum.	10 c. mm. of blood.	Axillary glands.		Inguinal glands.		Mesenteric glands.	Spleen.
										R.	L.	R.	L.		
197	♀	Two meals daily, July 23, 24, and 25, consisting of boiled rice and sterilised milk. One similar meal at 8 A.M. July 26.	Infected "whole" milk (Goat No. 114), 800 *M. melitensis* per c.c.	Each monkey supplied with 250 c.c. of its corresponding milk (see previous column) in open pannikin at 9 A.M., and a further 250 c.c. of milk at 11 A.M. July 27.	17	1 : 10	28	1 : 50	—	+	+	+	+	+	+
196	♀		Infected "whole" milk (Goat No. 115), 300 *M. melitensis* per c.c.		21	1 : 10	33	1 : 100	—	*	*	*	*	*	*
Controls.															
189	♂		Sterilised milk mixed (Goats No. 114 and 115).		—	—	28	—	—	—	—	—	—	—	—
190	♀				—	—	48	—	—	—	—	—	—	—	—

* Enlarged and tuberculous.

could be detected or recovered. While in this case there is not a shadow of doubt in the minds of the experimenters that the monkey had been successfully infected, the coexistence of abdominal tuberculosis had exercised such a profound influence on the course of the disease as to render the absolute proof of the existence of infection, viz., the recovery of *M. melitensis* from the various organs *post mortem* impossible to obtain, and this animal must consequently be eliminated from the experiment.

The second control, which was killed on the 48th day, showed no signs *post mortem* of infection with *M. melitensis* and at no period during the course of the experiment could specific agglutinins be detected in its blood serum.

The net result of this experiment, however, is still to conclusively prove that infection may result in the monkey (*e.g.*, No. 197), after the ingestion of one quantum of naturally infected milk, and that not a grossly contaminated sample, with an incubation period of about 17 days.

Series V.—In the next experiment an attempt was made to reproduce the conditions obtaining in the case of a hospital, barracks or other large institution supplied with goats' milk by a contractor who would necessarily command the daily milkings of a large number of goats and who would, for the convenience of all parties, supply "mixed" milk in churns of several gallons capacity.

Experience based upon the examination of the milch goats in a large number of herds from all parts of the island had already shown that a typical herd consisted of something like 60 per cent. normal goats yielding milk free from contamination with *M. melitensis*; 30 per cent. of goats previously infected (and whose milk yields a definite agglutination reaction), but which are either completely convalescent or else are in an early stage of the disease and are consequently not discharging the specific micro-organism in the milk; and 10 per cent. of goats whose milk not only gives a strong agglutination reaction, but also contains *M. melitensis* in varying numbers.

The infective material in this experiment was consequently made up in the following manner:—

Three perfectly healthy goats which gave no evidence of having been infected with *M. melitensis* were milked to the extent of 1 litre each into a sterile bottle.

Goats Nos. 101, 108, 110, whose milk gave a good agglutination reaction, but although plated regularly several times a week for some months had never yet yielded *M. melitensis*, were milked, also in sterile bottles, to the extent of 500 c.c. each. Goat No. 117, whose milk gave a good agglutination reaction and invariably yielded *M. melitensis* when plated, in numbers ranging from a few hundreds to thirty thousand and upwards per cubic centimetre, was milked to the extent of 500 c.c. into another sterile bottle.

Taking 500 c.c. as the unit of measurement, two volumes of milk from each of the three first goats were mixed with one volume from each of Goats Nos. 101, 108, 110 and 117, thus forming a fairly representative "mixed" milk such as would be obtained from an average herd.

Before the mixing was actually carried out, centrifugalised samples or suitable dilutions from the milk of each goat were plated out, and after the usual period of incubation and observation the following results were obtained:—

Goat 117.	Agglutination reaction positive.	Contained approximately 1000 *M. melitensis* per c.c.
,, 101.	,, ,, ,,	Contained no *M. melitensis*.
,, 108.	,, ,, ,,	,, ,,
,, 110.	,, ,, ,,	,, ,,
,, 2.	,, ,, negative	,, ,,
,, 3.	,, ,, ,,	,, ,,
,, 8.	,, ,, ,,	,, ,,

Samples of the "mixed" milk plated after mixing gave an average of 100 *M. melitensis* per cubic centimetre.

Two hundred and fifty cubic centimetres of this "mixed" milk were supplied to each of five experimental monkeys at 9 A.M. It was impossible, however, to see what was going on inside the mosquito-proof cubicles and, as much of the milk was wilfully wasted by the animals, a further quantity of 250 c.c. was filled into each monkey's pannikin at 11 A.M. At one o'clock the pannikins were removed, sterilised and refilled with a feed of boiled rice. From this point onwards the ordinary method of feeding was resumed and no more infective material was administered.

Of this batch of five animals, one (191) was found a few days after the feeding to be prostrate and helpless. It refused all food, ran a markedly intermittent temperature, and was found dead on the 10th morning. No serum reaction had been exhibited by this animal during its lifetime, and *post-mortem* examination failed to reveal infection by *M. melitensis*, or indeed any obvious cause for death. This animal must consequently be disregarded and is not included in the table dealing with this experiment.

Of the remainder, Monkey No. 195 first yielded a positive serum reaction (1 : 10) on the 17th day, Monkey No. 194 on the 18th day and Monkey No. 192 on the 21st day. The first Monkey (No. 195) pursued a remarkable course. From the 17th day onwards the dilution in which the serum reaction was obtainable steadily rose until on the 25th day it had reached 1 in 100. At the end of the third week blood was extracted from the external saphenous vein of each of the monkeys comprised in Series IV, V, and VI and planted in broth. Two days later all the tubes were found to be badly contaminated, with the

Table XVII.—Feeding Experiments in 1906.—Series V.

No. of monkey.	Sex.	Preparation for feeding.	Dose of infective material.	Method of administration.	Serum reaction.		Duration of experiment in days.	*Post-mortem* findings.							
									M. melitensis recovered from—						
					Day of appearance.	Amount of dilution.		Value of serum.	10 c. mm. of blood.	Axillary glands.		Inguinal glands.		Mesenteric glands.	Spleen.
										R.	L.	R.	L.		
195	♀	Two meals daily, July 23, 24 and 25, consisting of boiled potatoes, boiled rice and sterilised milk. One similar meal at 8 A.M., July 26.	Not more than 50 c.c. of mixed milk from healthy and infected goats (Nos. 102, 103, 118, 101, 108, 110, and 117) containing 100 *M. melitensis* per c.c.	Each monkey supplied with 250 c.c. milk in an open pannikin at 9 A.M., and a further 250 c.c. at 11 A.M., July 27.	17	1 : 20	38	*	†	—	—	—	—	—	—
194	♀				18	1 : 10	31	1 : 50	+	—	—	—	—	—	+
192	♀				21	1 : 10	33	1 : 100	—	+	+	+	+	+	+
193	♂				—	—	48	—	—	—	—	—	—	—	—
Controls. 189	♂		About 50 c.c. sterilised goat's milk.		—	—	28	—	—	—	—	—	—	—	—
190	♀				—	—	48	—	—	—	—	—	—	—	—

* On the 25th day the serum from this monkey gave a positive reaction with a dilution of 1 in 100.

† On the 21st day the blood from this monkey yielded *M. melitensis* in small numbers.

exception of those planted with blood from Monkey No. 195. These tubes were plated out and yielded a scanty growth of *M. melitensis.* The serum reaction then fell rapidly and on the 32nd day an incomplete reaction was all that could be obtained with 1 : 20 dilution. When killed on the 38th day agglutinins appeared to be absent from the blood serum, as no reaction could be obtained even with 1 : 10 solution. Cultivations from the various organs remained sterile and the non-recovery of *M. melitensis*, *post-mortem*, would appear to indicate that the infection was of a very mild and transitory type.

Another Monkey (No. 193) showed no sign whatever of infection throughout the course of the experiment, no trace even of agglutinin was present in its blood serum up to the date of the termination of the experiment on the 48th day, when it was chloroformed and examined *post-mortem.* *M. melitensis* was not recovered from any of its organs. It is, of course, possible, but not probable, that the monkey refused to take any of the infected milk and the absence of infection may be due to this cause, or to absolute continuity of the mucous membrane of the upper portion of the alimentary tract, but these are pure speculations. The fact remains that this monkey did not become infected by *M. melitensis.*

The two remaining monkeys (Nos. 194 and 192) when killed on the 31st and 33rd days respectively, afforded abundant evidence of generalised infection, so that as the net result of this experiment it may be stated that three out of four monkeys became infected as the result of each ingesting a quantity of mixed milk, containing not more than 5000 *M. melitensis.*

The full details of the results obtained are given in tabular form (p. 62).

Series VI.—As in all the preceding experiments, it may be urged that infection took place not through the intact mucous membrane of the alimentary canal, but through some lesion, perhaps only microscopic in extent, of the mucous membrane of the mouth; in this experiment an attempt was made to differentiate between such and direct absorption through the presumably intact mucous membrane of the stomach and intestinal walls. Two monkeys were, therefore, selected and prepared for the experiment in a manner precisely similar to that adopted in the other experiments; Monkey No. 198 was carefully anæsthetised with an A.C.E. mixture (alcohol, 1 part; chloroform, 3 parts; ether, 6 parts) and when fully unconscious, his mouth gently opened and a wooden gag introduced and fixed behind the canine teeth. The tongue was then pulled forward and a No. 8 soft rubber catheter passed down the œsophagus into the stomach. A small funnel was inserted into the open end of the catheter and 45 c.c. of "whole" milk from Goat No. 117, containing 1000 *M. melitensis* per cubic centimetre poured through into the stomach. Half a minute later the catheter was washed through with 10 c.c. of sterile salt solution, and after another short interval the

Table XVIII.—Feeding Experiments in 1906.—Series VI.

No. of monkey.	Sex.	Preparation for feeding.	Dose of infective materials.	Method of adminstration.	Serum reaction.		Duration of experiment in days.	*Post-mortem* findings.							
									M. melitensis recovered from—						
					Day of appearance.	Amount of dilution.		Value of serum.	10 c. mm. of blood.	Axillary glands. R.	Axillary glands. L.	Inguinal glands. R.	Inguinal glands. L.	Mesenteric glands.	Spleen.
198	♂	Two meals daily, July 23, 24, and 25, consisting of boiled potatoes, boiled rice, and sterilised milk. One similar meal at 8 A.M. July 26.	45 c.c. infected milk from Goat No. 117, containing 1000 *M. melitensis* per c.c.	Through soft rubber catheter passed into stomach under an anæsthetic.	—	—	42	—	—	—	—	—	—	—	—
199	♂		45 c.c. infected milk from Goat No. 117, containing 4000 *M. melitensis* per c.c.		—	—	42	—	—	—	—	—	—	—	—
Controls.															
189	♂		About 50 c.c. each of sterilised goat's milk.	*Vide* Table XVII	—	—	28	—	—	—	—	—	—	—	—
190	♀				—	—	48	—	—	—	—	—	—	—	—

open end of the catheter was clipped and the instrument carefully withdrawn. On removing the pressure from the upper end of the catheter nothing but a few drops of clear saline solution flowed from the eye. The animal was kept under the influence of the anæsthetic for a further period of five minutes, then gradually allowed to come to. No regurgitation of milk took place and the monkey was soon sufficiently recovered to be returned to his cubicle. By similar means, Monkey No. 199 had 45 c.c. of whole milk from Goat No. 111, containing 4000 *M. melitensis* per cubic centimetre introduced into his stomach and in this instance also regurgitation of the food was absent.

Of these two animals, Monkey No. 199, which had received some 180,000 *M. melitensis*, showed no signs of the formation of specific agglutinins throughout the entire course of the experiment, and when killed and examined *post-mortem* on the 42nd day the cultivations prepared from the various organs remained sterile. Monkey No. 198, which had received 45,000 *M. militensis*, yielded on the 17th day a very incomplete reaction with 1 in 10 solution of blood serum. A similar incomplete reaction was noted in the same dilution on the 21st and 25th days. No further reaction was obtainable, and when the animal was killed on the 42nd day, the cultivations prepared from the various organs—like those from Monkey No. 199—remained sterile. This experiment therefore yielded completely negative results. In neither case did the introduction of fairly large numbers of *M. melitensis* into the stomach direct—avoiding any contact with the mucous membrane of the upper portion of the alimentary canal—result in the infection of the experimental animal. As the experiments comprised in Series IV, V, and VI were carried out on the same day and under exactly comparable conditions, Monkeys No. 189 and 190 have done duty as controls to all.

The details of this experiment are shown in Table XVIII (p. 64).

4. *Clinical Features of Food Infections in the Monkey.*

On the clinical aspect of the infection produced by these feeding experiments it is unnecessary to dwell at length or to reproduce temperature charts in detail. A few points of interest may, however, be briefly mentioned :—

Temperature.—Speaking generally, the infection, as judged by the course of the animal's temperature and supported by its general appearance and behaviour, appeared to be far from severe during the six or seven weeks some of the animals were under observation, but opinions based upon clinical symptoms were rudely contradicted by the result of the *post-mortem* examinations.

In this connection it is necessary to emphasise the ready response of the temperature of the monkey to environmental conditions. The normal temperature of the Rhesus when confined in the cool, quiet rooms of the Lazzaretto and attended by a man accustomed to handle such animals and thoroughly acquainted with their habits, averages about 101° F., although such extremes as 99°·2 in the morning and 103°·4 in the evening have been noted. (The temperatures are here given in Fahrenheit degrees for the sake of uniformity with the temperature charts recorded in previous parts of these Reports.) When transferred to the hot, sunny terraces outside the Laboratory—overlooking, perhaps, the noisiest street in Valletta—and handled by a new and ignorant attendant, who at first obviously went in fear of his charges, the normal temperature became probably half a degree higher. When, as sometimes happened, the animal was allowed to resist capture and to struggle for some minutes before it was sufficiently firmly held to allow of its temperature being taken, the resulting reading was sometimes as much as 2° F. too high. Under such conditions as these, febrile temperatures are apt to be fallacious unless very rigorously scrutinised, and to illustrate these irregularities of the temperature curve, the chart of one of the normal healthy controls—Monkey No. 172—may be utilised.

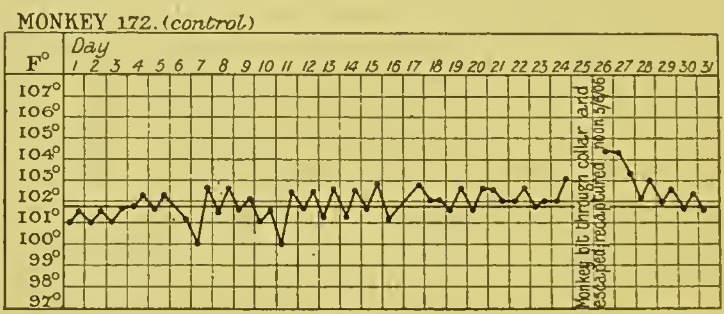

Chart 6.

Another point to be noted with regard to the even temperatures many of the experimental monkeys exhibited, is that, owing no doubt to the strictly enforced cleanliness of the monkeys and their habitations, and the minute attention devoted to the quality of their food, the cases were uncomplicated by diarrhœa and gastro-intestinal disturbances, which are, perhaps, the most fruitful causes of febrile reaction in the monkey.

Again, speaking generally, the temperature chart of the Rhesus infected with *M. melitensis*, except in the case of very severe infections such as follow intercranial injections of the micrococcus, shows but one period of pyrexia, followed by an intermittent temperature of slight range and short duration. A second period of pyrexia, or "wave" as it is colloquially termed, is quite the exception. The remittent type of pyrexia does, however, occur in the monkey; also this animal sometimes exhibits a type of temperature absolutely comparable to the one obtaining in man, when the subject of what Shaw has designated the "ambulatory" type of Mediterranean Fever.

Turning now to concrete examples, the temperature charts of Monkeys Nos. 153, 164, and 155 have been selected to illustrate the three types above referred to; and, although varying so widely in clinical aspect, the severity of the infection must have been of nearly equal intensity in these cases, judging by the results of the bacterioscopic examination, which showed that the blood and all the organs of these animals were literally teeming with the *M. melitensis.*

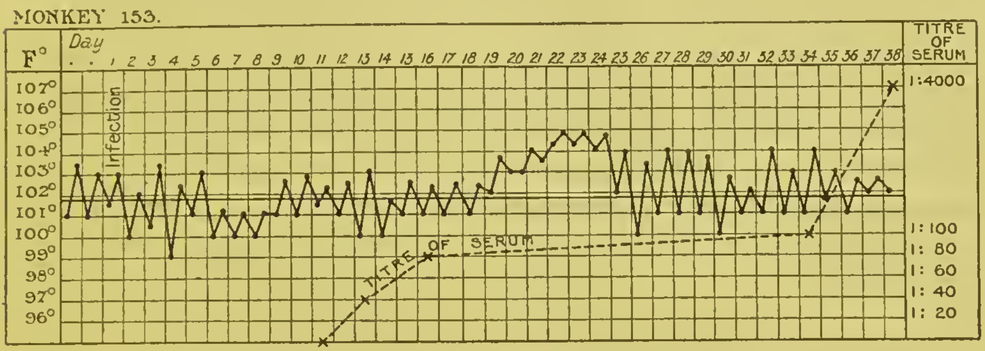

Chart 7.

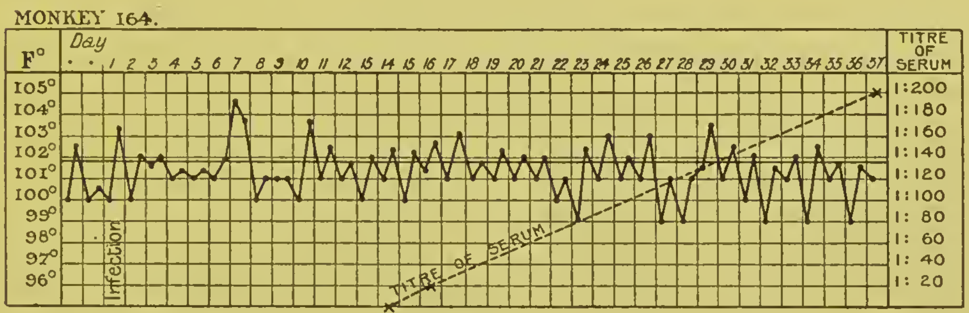

Chart 8.

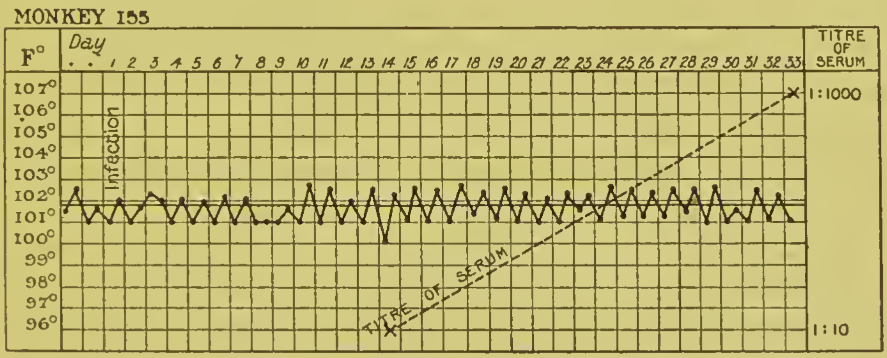

Chart 9.

Agglutination Reaction.—The repeated examinations that were made of blood from each of the infected monkeys showed that for a day or two, or even several days, before a definite reaction was obtainable, a 1 : 10 dilution of the serum produced what is regarded as an "incomplete" reaction—that is, the micrococci ceased to exhibit active vibratory movement and adhered together in small bunches, but large clumps and masses were not formed and the general field was made up of discrete cocci. Then a good reaction, large clumps in a perfectly clear fluid, readily visible with the two-thirds lens or, indeed, the unaided eye, would be produced by a low dilution of the serum—1 : 10 or 1 : 20. Very often, even at this stage, the macroscopical reaction in the sedimentation tube was absent. Next, the microscopical reaction would often disappear for a day or two, or even longer; finally, it would become firmly established and obtainable in the majority of cases in considerably higher dilutions, and the micro- and macroscopical reactions would control and confirm each other with absolute precision. The exigencies of experiment necessitating the destruction of animals early in the course of the disease are responsible for the fact that but few examples of the development of a very high agglutinative power in the serum were noted.

5. *The Action of Hydrochloric Acid and of Artificial Gastric Juice on* M. melitensis.

During the progress of these experiments, an investigation into the action of hydrochloric acid alone and also of artificial gastric juice upon the *M. melitensis* was undertaken. In the first instance, 1-per-cent. solution of hydrochloric acid to the amount of 5 c.c. was mixed in a sterile test-tube with a like volume of emulsion, in normal saline solution, of cultivation of *M. melitensis*, the emulsion being approximately of the strength of 1 milligramme of cultivation to 100 c.c. of saline solution, or, expressed in individual cocci, about 2,000,000 of *M. melitensis* per cubic centimetre; 5 c.c. of the emulsion mixed with an equal quantity of 0·10 per cent. hydrochloric acid were put up in a second tube, 5 c.c. of the emulsion of *M. melitensis* mixed with an equal quantity of 0·05 per cent. hydrochloric acid solution were put up in a third tube, and a control was prepared by mixing 5 c.c. of normal saline solution with 5 c.c. of the emulsion in a fourth tube. Immediately after mixing, a loopful of the contents of each tube was plated out and the tubes transferred to the incubator at 37° C. At intervals of 5, 10, 15, 20, and 30 minutes, and also after one and two hours' incubation, further similar plates were prepared from the mixture in each tube, and, after the usual period of incubation and observation, each of the plates was carefully studied. Many variations were made in the percentage strength of the hydrochloric acid solution and in the number of cocci per cubic centimetre of the emulsion, and the mixtures were similarly tested. The results obtained were fairly uniform and showed that when the very large numbers of cocci were suspended in weak solution of the acid, very little lethal action was demonstrable.

On the other hand, it will be seen from the experiment tabulated below, which is a typical one, that *M. melitensis*, when present in an aqueous solution of the acid in moderate numbers only, while able to withstand the lethal action of a 0·025-per-cent. solution of hydrochloric acid as well as of 0·05-per-cent. solution for considerable periods, is destroyed by 0·5-per-cent. solutions within an hour:—

Table XIX.—The Action of HCl upon Watery Suspensions of *M. melitensis*.

Composition of mixture:— Bacterial emulsion and solution of HCl.	Percentage of HCl in mixture.	Time of contact.			
		Immediately.	45 mins.	60 mins.	2 hrs.
5 c.c. + 5 c.c. of 1 per cent.	0·5	+	±	–	–
5 c.c. + 5 c.c. of 0·1 per cent.	0·05	+	+	+	±
5 c.c. + 5 c.c. of 0·05 per cent.	0·025	+	+	+	+
Control.					
5 c.c. emulsion of cocci + 5 c.c. of saline solution	–	+	+	+	+

+ = Good growth, ± = Scanty growth, – = No growth.

Next, a couple of sterile flasks were taken and 25 c.c. of milk from Goat No. 115, containing some 2500 *M. melitensis* per cubic centimetre, was introduced into the interior of one, and 25 c.c. of artificial gastric juice added and thoroughly mixed. The composition of the artificial gastric juice was:—

Pepsin	0·32 gramme
Hydrochloric acid	0·02 ,,
Sodium chloride	0·22 ,,
Sterile distilled water	100 c.c.

A control was formed by mixing 25 c.c. of milk from Goat No. 115 and sterile saline solution.

After plating a loopful from each mixture to determine the number of micrococci present at the commencement of the experiment, the flasks were placed in the incubator at 37° C. At 15, 30, 60 minutes, 2 and 24 hours, the flasks were removed from the incubator and about one-tenth of a cubic centimetre from the contents of each plated out, and after incubation the colonies of *M. melitensis* which developed were enumerated.

One of the experiments—a representative one—is tabulated below, and shows that the artificial gastric juice exerts some, although slight, inhibitory action on the growth of *M. melitensis* from the moment of contact.

Table XX.—The Action of Artificial Gastric Juice upon *M. melitensis* present in Naturally Infected Milk.

Mixture.	Time of contact.				
	Immediately.	15 mins.	30 mins.	60 mins.	2 hrs.
25 c.c. artificial gastric juice + 25 c.c. milk from Goat No. 115	420	640	130	250	440
Control.					
25 c.c. normal saline solution + 25 c.c. milk from Goat No. 115	1390	850	2700	1890	3050

The numbers give the calculated yield of *M. melitensis* per cubic centimetre.

III.—*M. melitensis* INFECTION FOLLOWING THE INGESTION OF INFECTIVE MILK IN MAN.

As an interesting and highly instructive corollary to the foregoing infections in the monkey, resulting from experimental feeding with infective milk, the occurrence of an epidemic of Malta Fever on board the s.s. "Joshua Nicholson," a cargo steamer, which conveyed a herd of milch goats from Malta to Antwerp towards the end of the summer of 1905, may be cited. Many of the officers and crew partook of the milk of these goats, and subsequent bacteriological investigations proved that some of these animals were infected by *M. melitensis*. As a result of the investigations made at the end of 1905 and during 1906, there can be no reasonable doubt that the cases of Malta Fever reported from the ship were due to the ingestion of the infected milk.

The history of the outbreak has been carefully compiled by Staff-Surgeon Clayton, and full epidemiological details, together with the history of each individual case so far as can be ascertained, is presented in a succeeding Part of these Reports, but because the history, read in the light of the results recorded in the previous section, savours so strongly of a carefully planned laboratory experiment, a short *résumé* is inserted here.

Résumé OF THE OUTBREAK OF MEDITERRANEAN FEVER ON BOARD THE S.S. "JOSHUA NICHOLSON."

1. *History of the Goats.*

Mr. Thompson, of the United States Bureau of Animal Industry, visited Malta in the summer of 1905, and during a stay of some months gradually purchased a herd of 61 milch goats (all healthy in appearance and good milkers, many being prize animals), and four billy goats. These he shipped on board the cargo steamer "Joshua Nicholson," on August 19, 1905, for passage to the United States *viâ* Antwerp. During the voyage, which lasted until September 2, 1906, when Antwerp was reached, the goats were milking well, and many of the ship's company partook freely of the milk—the officers drinking "mixed" milk collected in a large vessel, the members of the crew each obtaining "whole" milk from one goat in his own separate pannikin.

On arrival at Antwerp the goats were at once transferred to the quarantine station, where they remained for the five days that elapsed before they were re-embarked on the s.s. "St. Andrew" bound for New York, and during this voyage a large quantity of milk was again available for consumption. New York was reached about September 24, and the animals were transferred to the quarantine station at Athenia, N.J., where they remained under observation. Subsequent bacteriological examination resulted in the recovery of *M. melitensis* first from the milk of two of the goats and afterwards from that of several more.

2. *The Incidence of Mediterranean Fever among those who partook of the Milk.*

(a) *In the s.s. "Joshua Nicholson."*—In addition to four passengers (Mr. Thompson and three goatherds) present on the voyage from Malta to Antwerp, the "Joshua Nicholson" carried 23 officers and men. Of the crew of 19, the carpenter, boatswain, and mess-room steward, together with eight others (11 in all), left the ship at Antwerp; the boatswain was afterwards in hospital suffering from hernia; the movements of the remainder cannot be traced. Of the 12 remaining officers and crew, eight fell sick at intervals varying from 18 to 34 days from the embarkation of the goats, and in the cases of five of these eight the blood reactions leave no room for doubt that Mediterranean Fever was the cause of their illness.

The four members of the ship's strength who did not show any signs of illness were the second mate and the cabin boy, with whom the milk disagreed and who consequently had but very little, and two engineers (Germans) who drank the milk, it is true, but appear to have always boiled it.

Of the three goatherds, one (the chief goatherd) had undoubtedly been infected with *M. melitensis* previous to July, 1906, as evidenced by the presence of specific agglutinins in his blood, but whether recently or remotely it was impossible to say: about the two assistant goatherds no information could be obtained.

(b) *At Antwerp.*—The staff of the quarantine station and many individuals in the neigbourhood are said to have partaken of the milk, both raw and boiled, during the five days the goats were interned here, but no information can be obtained of the subsequent occurrence of cases of illness resembling Mediterranean Fever.

(c) *In the s.s. "St. Andrew."*—The s.s. "St. Andrew" carried 30 cattle men and the three goatherds and Mr. Thompson, in addition to a crew of 30 men. Most of these drank of the milk, but the master of the ship and also his owners state that none of the men suffered from any illness.

(d) *In America.*—With the exception of Mr. Thompson, who died in January, 1906, from "bilateral pneumonia following influenza," and about whose medical history, *quâ* Mediterranean Fever, no evidence can be obtained, only one person—a woman at the quarantine station—took the milk in any quantity. She, however, drank the mixed milk from several goats for a considerable period, and in December, 1905, suffered from a typical attack of Mediterranean Fever.

3. *The Results.*

In summarising the result of this unpremeditated experiment, several factors have to be considered. For instance, a certain unknown number of goats—more, however, than two—were shown to be secreting infective milk after their arrival in America, some three months after leaving Malta, but there is no direct evidence as to the number whose milk contained *M. melitensis* during the voyage, in summer weather, from Malta to Antwerp. Arguing from analogy with average Maltese herds, at least six should have been secreting infective milk. The goats purchased by Mr. Thompson were, however, picked animals and heavy milkers, and as experience has shown that the goats yielding the most milk in any given herd are the most likely to be passing *M. melitensis* in their milk, the probability is that in this particular herd of 60 milch goats (one having died the day after leaving Malta) the milk from considerably more than six was heavily infected—an inference which receives confirmation from the fact that the three officers and the steward who drank "mixed" milk each developed an attack of Mediterranean Fever, the remaining officer and the cabin boy, with whom the milk disagreed and who consequently did not drink it, remained well.

The members of the crew, on the other hand, each drank "whole" milk from a single goat, and apart from the possibilities of the milk being supplied on any particular occasion from an uninfected animal, a reference to Section I (3), shows clearly the possibilities of a man who obtains milk, even from an infected animal, avoiding the ingestion of *infective* milk.

Apart from such considerations, however, it suffices to state the net result as follows:—

Of 23[*] men on board the s.s. "Joshua Nicholson" who drank on one or more occasions presumably infected milk, no evidence whatever is available as to 12 and no relevant information as to Mr. Thompson; of the remaining 10, one suffered from hernia only, one was infected by *M. melitensis* at an unknown date, while eight suffered from febrile attacks—5 (or 50 per cent. of them) yielding conclusive evidence of infection by *M. melitensis.*

IV.—The *Rôle* of the Mosquito and other Blood-sucking Insects in the Dissemination of *M. melitensis.*

That the mosquito may act as the vehicle in the conveyance of the infection of Mediterranean Fever may be an example of the wish being father to the thought, and due in part to the inconvenience caused to residents in Malta by these little pests. The rise in the case-incidence curve of the disease in June, July, August and September,[†] roughly corresponding as it does to the mosquito season, gives colour to a suggestion which is by no means new. In 1902 Zammit produced detailed evidence of an epidemiological character in support of the mosquito theory—that is to say before this observer had noted the natural infection of goats—later on Zammit, Horrocks and Kennedy produced experimental evidence of similar tendency, and more recently still Ross has laboured the point on purely theoretical grounds.

Before, however, attacking the question of the conveyance of *M. melitensis* infection, it became necessary to study the species of mosquitoes prevalent in Malta, since it seemed possible, arguing from analogy, that some one particular species, and one only, acted as the vehicle.

1. *Species of Mosquitoes occurring in Malta.*

Six species, representing five genera and two sub-families, are of common occurrence, viz.:—

Culex fatigans	Sub-family Culicina.
Culex pipiens	
Theobaldia spathipalpis......	
Acartomyia Zammitii	
Stegomyia fasciata	
Anopheles maculipennis......	Sub-family Anophelina.

and apart from Anopheles, which is restricted at present, so far as breeding place is concerned, to one valley in the centre of the island,

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[*] That is disregarding the two men who boiled the milk before drinking it, and the officer and cabin boy who did not drink the milk.

[†] Johnstone, these Reports, II, p. 36.

Table XXI.—Synopsis of the Characters of the Maltese Mosquitoes.

	I and II. *Culex fatigans* and *C. pipiens.*	III. *Theobaldia spathipalpis.*	IV. *Acartomyia Zammitii.*	V. *Stegomyia fasciata.*	VI. *Anopheles maculipennis.*
Distribution	Over the entire island. Essentially a domestic mosquito.	As I and II.	Round the entire coast-line. On first appearance is found also in houses in urban districts.	As I and II.	Restricted to the Wied Ta Klighi, a deep valley between the Imtarfa and Klighi Hills.
Breeding places	Shallow stagnant pools; agricultural water-tanks; any small collections of stagnant water in artificial receptacles, such as flower-pots and saucers, buckets, old tins, etc., near to human habitations.	As I and II. Also in slowly flowing watercourses in rural districts.	In the salt pans on the rocky coast. In salt-water pools along the shores of the harbours.	As I and II. Often found in the same collection of water.	In the watercourse occupying the bed of the ravine from Fiddian Bridge to the artificial dam.
Time of appearance	Found all through the year.	As I and II.	April to October.	June or July to September.	May or June to September.
Most plentiful	From May to July onward to September.	May to August.	After October the heavy seas produced by the Grigali wind cause the disappearance of this mosquito.	July and August.	After September the watercourse is full and swiftly running.
OVUM—					
Length	0·7 to 0·9 mm.	1 mm.	0·5 mm.	0·4 to 0·6 mm.	0·7 mm.
Shape	Oblong, slightly curved; one end rounded, the other somewhat pointed.	As I and II.	Pointed oval.	Ovoid, more pointed at one end than other. Surrounded by a series of small air-chambers, which give a reticulated appearance to surface.	Elliptical.
Colour	White when first laid, rapidly turning grey, brown, and finally black.	As I and II.	White when first laid, rapidly becoming dark brown and black.	As I and II.	White, rapidly turning to greyish black.
How deposited	In masses or rafts irregularly quadrilateral, or elongated spindle-shaped; 5—8 mm. long × 3—4 mm. broad. Upper surface of raft slightly concave, as eggs, which cohere by sticky lateral surfaces, are arranged pointed ends upwards.	Rafts larger than I and II; pointed oval or boat-shaped, 10—15 mm. long by 5—8 mm. at broadest point.	Usually in rows, side by side, like a palisade, rarely singly or in pairs.	Singly and in pairs, side by side.	Singly, but float close to each other, and often cohere by their ends and form triangular patterns.
Number	200—400.	100—200.	25—100.	40—150.	40—100.
When deposited	Soon after sunrise, and also at dusk.	As I and II.	As I and II.	As I and II. Also at mid-day.	As I and II.
Period of incubation	24—72 hours. The larvæ escape through the thin rounded lower ends of ova.	As I and II.	As I and II.	48 hours to 3 days.	As I and II.
LARVA—					
Size of adult	4 mm. long.	10 mm. to 12 or 14 mm. long.	5 mm. long.	6 mm. long.	5—7 mm. long.

Colour	Pale grey or straw-coloured with greenish tinge, or deep dirty brown.	Pale greenish to greenish brown.	Grey to light brown.	Greyish white.	Yellowish green to green or black, with mesial dorsal dark stripe.
Head	Large, with very prominent dark eyes.	Bright chestnut-brown, with black eyes, and band across nape.	Quadrangular, prominent oval eyes.	Large and quadrangular, smaller than thorax.	Small, oval in shape, with many black spots.
Thorax	Larger than head.	Wider than head.	Larger than head; three tufts of bristles on either side.	—	Broader than head.
Abdomen	Nine segments, four anal fins at apex of ninth.	—	All nine segments nearly the same width. Segments 1 to 8 bear lateral tufts of long bristles. Segment 9 bears four anal papillæ at apex and ventral tufts of bristles arranged fanwise.	All nine segments nearly the same width. Anal fins short, bluntly finger-shaped.	Nine segments progressively decrease in width, but increase in length. Segments 1 to 3 bear long lateral bristles, which balance larva horizontal to surface film. Joint 9 bears swimming fan formed of long feathered bristles. Segment 9 carries four anal papillæ only.
Respiratory tube	Long, rising from eighth segment.	Short and thick.	Long, rising from 8th segment.	Short, broad, barrel shaped, and black in colour.	Two simple spiracles only on 8th segment.
Position when breathing	Hangs obliquely or vertically, head downwards, top of siphon at surface film.	As I and II.	Obliquely, head downwards or horizontally just below surface film.	At small angle to surface film, or horizontal. Remains at bottom of water for long periods.	Horizontal immediately below the surface film.
Duration of larval stage	Moults three or four times during a period of 14—21 days.	As I and II.	11—15 days.	8—12 days.	16—21 days.
PUPA—					
Size	6 to 8 mm.	8 to 10 mm.	5—6 mm.	7 mm.	7 mm.
Colour	Dirty brown.	Dirty brown.	Dark brown.	Deep brown.	Yellowish green to grass-green, or dull brown.
Head and thorax	Fused.				
Eyes	Large, oval, prominent.	—	Oval and prominent.		
Respiratory horns	Two trumpet-shaped, narrow bases, oblique openings rise from dorsum of thorax.	Curved with truncated ends.	Curved, funicular, narrow bases, oblique openings.	Large, broadly cylindrical, obliquely truncated at apex, narrowed at base.	Short with square truncated ends.
Abdomen	Nine segments, flattened dorso-ventrally. 8th segment carries pair of oval fins, supported on hard mid-rib. 9th segment carries blunt process on either side of anus.	1st segment, tree-like tuft of bristles on dorsum. 9th segment, prominent anal fins.	As I and II.	1st segment, tuft of bristles. Two broad anal fins with long central rod.	Segments 4 to 8, black spot on either side.
Duration of pupal stage	Imago emerges in from 2 to 4 days.	As I and II.	1—3 days.	2—3 days.	5—10 days.

Table XXI—*continued.*

	I and II. *Culex fatigans* and *C. pipiens.*	III. *Theobaldia spathipalpis.*	IV. *Acartomyia Zammitii.*	V. *Stegomyia fasciata.*	VI. *Anopheles maculipennis.*
IMAGO—					
Size	♀ 4·5 to 5·5 mm.; ♂ slightly smaller, 4—5 mm.	9—11 mm.	4—4·5 mm.	3—5 mm.	5—7 mm.
Head	Brown, covered with pale golden brown curved scales, with scattered dark brown upright forked scales.	Almost black, with two median curved lines uniting in front and behind as a white line between the eyes.	Ochraceous yellow at the sides. White in the middle.	Dark brown or black, with white patch in the middle and white patch on either side.	Black, with two patches of creamy scales, divided by a central line.
Thorax	Brown, with curved scales; two parallel dark lines and three rows of black bristles. Ornamentation very variable.	Umber brown with white lines and spots.	Brown with yellowish and creamy curved scales.	Dark reddish brown, with two median parallel pale lines and a curved silvery line on each side.	Brown, bluish gray in centre, deep brown at sides.
Abdomen, dorsum	Dark brown scales, with flaxen basal bands, broader laterally to form spots.	Yellow scales with scattered black scales.	Blackish brown with basal white bands.	Black, with white basal bands and lateral spots.	Dark brown, with tawny brown markings and dark apical bands.
,, venter	Pale yellow scaled.	Pale flaxen scales with few scattered dark ones.	All creamy.		
Legs	Dark brown and unbanded; bases of coxæ and femora pale; knee-spot white or yellow; tarsi dark brown; ungues, ♀ equal and simple, ♂ fore and mid unequal and toothed, hind equal and simple.	Coxæ yellowish with white scales. Femora pale at base with ragged yellowish band before apex; tibiæ striped black and white; metatarsus black with white basal band; tarsi black; ungues equal and simple.	Brown apical and basal pale bands. The last hind tarsal white. Ungues equal and simple.	Black with basal white bands. Last segment of hind legs pure white. Fore and mid ungues toothed; hind untoothed.	Unbanded, brown. Small pale knee-spot, tarsi dark brown, ungues equal and simple.
Wings	1st sub-marginal cell longer and narrower than 2nd posterior cell. *C. fatigans*, ratio stem to cell = 1 : 4. *C. pipiens* ,, ,, = 1 : 7.	Curved, with long brown scales aggregated to form three distinct dark spots, one where the 2nd long vein rises from the 1st, one at the anterior and mid cross veins, and one at the fork of the 5th long vein.	Mottled brown and grey scales.	Veins clothed with long, narrow, brown scales.	Covered with narrow black scales aggregated to form four spots, one at the base of the first fork cell, one at the second fork cell, one at the cross veins, and one at the base of the second long vein.

[I am much indebted to Mr. F. V. Theobald, F.E.S., for his kindness in reading through the proof of this table, and supplying data for some of the then blank spaces.—JNO. EYRE.]

and Acartomyia, which breeds along the coast line, these species are fairly uniformly distributed over the entire island.

The accompanying synoptical table, adapted from Theobald in accordance with observations made in Malta during the summer of 1906, details the chief points of similarity and distinction between the various mosquitoes, and is introduced here in the hope that it may prove helpful to workers in Malta in this or similar fields.

2. *Supply of Mosquitoes and Notes on their Habits in Captivity.*

The supply of insects required for the experimental work in connection with the attempts to transmit *M. melitensis* infection by the aid of mosquitoes was kept up by repeatedly collecting large quantities of larvæ and pupæ, transferring them to shallow glass dishes of water and allowing them there to complete their development—each species being confined in a separate gauze covered breeding-cage provided with two soft gauze sleeves (of sufficient capacity to admit the hand and arm), situated in the front of the cage and so permitting ready access to any part of the interior. To facilitate the entry of the hand, the free extremity of the long sleeve was attached to a circle of wire. By twisting the sleeve upon itself and pushing the wire circle under a tightly stretched piece of elastic (attached to the front of the cage) the imagines were effectually prevented from escaping (fig. 3). With adequate care, in such a cage the mortality of imagines when they emerged from their pupa cases was by no means excessive, and after being fed on blood the impregnated females readily laid their eggs in the water in which they had passed the early stages of their existence.

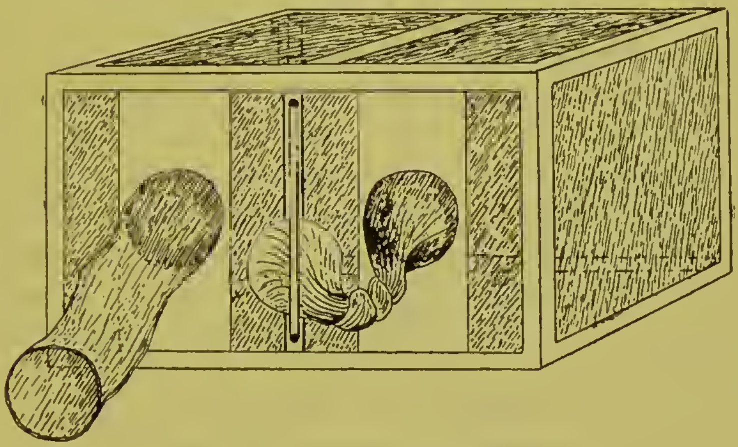

Fig. 3.—Mosquito Breeding Cage.

The morning of the day preceding a feeding experiment the perfect females were captured in detail and each transferred to a short length of glass tubing (4 to 6 cm. long by 1·5 cm. in diameter) covered at one end with a piece of muslin secured by an indiarubber band and closed at the other by a well-fitting cork, where they remained, without food, for from 30 to 32 hours. This useful little piece of apparatus (fig. 4) enabled a distinguishing number or letter to be assigned to every mosquito, simplified the handling of the insects, and rendered it an easy matter to ascertain that feeding had actually taken place, and indeed to watch the entire progress of the act.

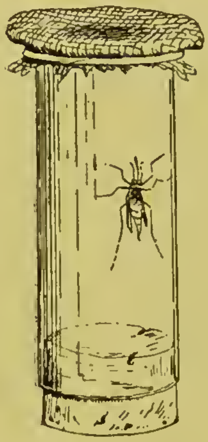

Fig. 4.—Experimental Feeding-tube for Mosquitoes.

In connection with these experiments many difficulties, varying with the species, were encountered, some of which may be usefully referred to.

In the first place, cannibalism is a marked feature of mosquito life during the larval stage, of all the species, and probably not more than 50 per cent. of the larvæ—under laboratory conditions—attain maturity. The occasional addition of a few grains of tapioca to the water containing the larvæ certainly reduces, but by no means prevents, this wastage.

Then it was soon found that female Acartomyia and sometimes *Culex* (*fatigans* and *pipiens*) when segregrated immediately after emerging from the pupa were weakly, could not under any conditions be induced to bite, and soon died. This reluctance of the virgin to suck blood has frequently been noted, and it was consequently found necessary to allow the females to remain in company with the males for two or three days after emerging from the pupa in order to ensure an adequate supply of impregnated females, the insects being freely supplied with fruit—chiefly apples—and syrup during this period. By adopting this method the weakly imagines, which quickly died, were weeded out and a supply of sturdy females was insured.

Again, Acartomyia, although naturally a voracious feeder during the daytime as well as after dusk, like Culex could not in captivity be induced to bite until after dusk and occasionally until after night was well advanced. Captive Stegomyia, on the other hand, bit readily at any time during the 24 hours.

In feeding mosquitoes a large fund of patience is required. Some, particularly Stegomyia, will go down as soon as applied to the skin and commence feeding at once; others, like Acartomyia, are a considerable time testing area after area of skin with the proboscis before a suitable spot is found and the actual abstraction of blood begins. Individuals again may have to be coaxed before they will begin operations by shading the tube in which they are confined from the direct light, or by raising its internal temperature by closely encircling it with the hand.

Having commenced to feed, the time taken by various individuals to completely fill themselves varies largely and bears no direct relationship to the size of the insect. A presumably thirsty Stegomyia will sometimes fill itself inside 30 seconds, another of apparently equal capacity, or the same individual on another occasion will require some 15 minutes to reach the stage of repletion: Acartomyia usually requires from one minute to five minutes to complete the meal. The shortest time noted was achieved by one of the largest Stegomyia in our possession, which required only 15 seconds to appease its appetite.

The quantity of blood taken up by the insect at one feed is also a matter of some importance, for the minimal quantity of blood from Malta Fever patients in which Gilmour and Shaw* were able to demonstrate the presence of *M. melitensis* was approximately 3 and 4 cubic millimetres respectively. The micrococcus may, however, be present in the blood of human patients in much greater numbers than these figures would seem to indicate (*vide* p. 125), as shown by the examination of the blood in a small series of cases this summer, when *M. melitensis* was once found to the number of 10,000 per cubic centimetre—a quantity of infective material in the blood stream comparing with that found in mild cases of malaria or very severe cases of streptococcic septicæmia.

Estimations were therefore made by weighing one of the empty mosquito tubes, transferring a captured mosquito to its interior and again weighing it—the difference being taken to represent the weight of the insect. The mosquito was immediately fed and again weighed, the difference in the weights of tube *plus* mosquito, before and after feeding, being regarded as the weight of the blood ingested, and may fairly be taken as sufficiently accurate for practical purposes. From a series of eight such weighings the average quantity of blood taken up by Stegomyia was 3 milligrammes, the extremes noted being 1·5 milligrammes and 5·5 milligrammes. *Stegomyia fasciata* was the species selected for the purpose of this experiment, on account of the readiness with which this insect bites even during the day time. Thus all the operations of weighing, feeding, and again weighing could be carried out out during the middle of the day, when the air temperature was at its maximum, and the air humidity, which is an important factor in Malta, where delicate weighings are involved, at its lowest point. The details of these weighings are given in tabular form below.

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* *Vide* these Reports, I, p. 10, and III, p. 19.

The amount of blood taken by a mosquito bears relationship to the elasticity of the skin of the abdomen rather than to the size of the insect, for of the two extremes noted the maximum, 0·0055 gramme blood, was ingested by a very small female—much smaller, in fact, than its weight (empty) would indicate.

Table XXII.—Showing Average Weight of *Stegomyia fasciata* before Feeding, and of Blood Ingested when the Insect was filled to Repletion.

No.	Weight of fasting mosquito.	Weight of mosquito *plus* blood—after feeding.	Weight of blood ingested at one meal (deduced from difference in weights given in Cols. II and IV).
	gramme.	gramme.	gramme.
1	0 ·0020	0 ·0035	0 ·0015
2	0 ·0020	0 ·0055	0 ·0035
3	0 ·0020	0 ·0065	0 ·0045
4	0 ·0030	0 ·0072	0 ·0042
5	0 ·0030	0 ·0085	0 ·0055
6	0 ·0080	0 ·0105	0 ·0025
7	0 ·0020	0 ·0045	0 ·0025
8	0 ·0010	0 ·0035	0 ·0025
Average	0 ·00262	0 ·00621	0 ·00333

3. *The Duration of Life of* M. melitensis *in the Body of the Mosquito.*

Since the species of mosquito common to the Island as a rule only feed upon the human subject at intervals of 48 hours, the first and most important points for consideration were (1) whether the mosquito would serve as a host for the micro-organism, and if so (2) the duration of life of the *M. melitensis* in the insect's body.

Previous workers* had isolated *M. melitentis* from the bodies of four mosquitoes (captured in the wards of hospitals where they had had opportunities of biting patients suffering from Malta Fever as well as patients suffering from other diseases, and also normal individuals), out of a total of 450 which they had dissected, and therefore disregarding the possibility of accidental external contamination of the mosquitoes, it was assumed to be proved that the mosquito could act as a host for *M. melitensis*, thus disposing of the first point.

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* Horrocks and Kennedy, *vide* these Reports, IV, p. 72.

With regard to the second point, the simplest method of determining the duration of life of the micro-organism within the insect's body would have been to feed a large number of mosquitoes simultaneously upon a patient suffering from Mediterranean Fever in whose blood *M. melitensis* was known to exist in large numbers; then to dissect several mosquitoes at regular intervals and prepare plate cultivations from the contents of the stomach, etc. The difficulties in carrying out such an experiment were great. Cases of Mediterranean Fever in the wards of the Military and Naval Hospitals were few in number and mild in character; moreover, Gilmour and Shaw had stated that the micrococcus could only be isolated from comparatively large quantities of blood, and that its presence was by no means constant. On humanitarian grounds, too, it did not appear justifiable to subject any one patient to the discomfort of being thus bitten by scores of mosquitoes in the prosecution of what were, after all, but preliminary observations.

Advantage was, therefore, taken of the fact that an extremely acute septicæmia, associated with the presence of numerous cocci in the peripheral circulation, can readily be induced in the guinea-pig by means of intracerebral inoculation of highly virulent cultivations of *M. melitensis*,* consequently this animal was substituted for the human subject and, with this modification, the experiments suggested above were initiated.

The procedure adopted was as follows :—

At 8 or 9 A.M., two or three guinea-pigs were successively anæsthetised and injected intracranially with 0·1 of a loop of a 24-hour-old culture of the highly virulent culture emulsified in 0·05 c.c. Advantage was taken of the insensibility of the animal to epilate a fair-sized area of the skin of the back between the shoulders. The temperature of the animal was then carefully watched. Usually, five or six hours later, or by 5 P.M. at latest, the temperature had reached 105° or 106° F., and was maintained at this level for many hours; with such a temperature it was known, as a matter of experience, that the circulating blood contained numerous cocci. The mosquito tubes already described, each containing an impregnated female mosquito, were then applied in turn, gauze covered end downwards, to the guinea-pig's back over the area of skin previously denuded of hair, and the insects allowed to fill themselves with blood. Immediately the entire batch of insects had fed, several insects were killed by ether vapour, placed in sterile Petri dishes and dissected at once. The bloody contents of the stomach were removed and emulsified with a small quantity of sterile salt citrate solution (0·75-per-cent. sodium chloride and 1-per-cent. sodium citrate in distilled water) and distributed over the surface of three or more nutrose-agar plates. After incubation, the colonies of *M. melitensis* that had developed were enumerated and their identity verified. This process was repeated at regular intervals, two or three insects being destroyed every 24 hours or so, and the contents of their stomachs plated, the unused insects being kept in a cage containing a vessel of water in which to lay their eggs, and supplied with fresh fruit, or retained in the separate feeding-tubes into which small cubes of apple or other fruit were introduce ddaily. In this way it was determined that the numbers of *M. melitensis* usually decreased as time went on, the greater number being voided with the droppings in the first 48 hours after feeding, but that in one or two instances the cocci appeared to actually multiply in the mosquito's stomach for a day or two, while it was certainly proved that living micrococci could be demonstrated in the interior of the mosquito four days after feeding on an infected animal.

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* Eyre, *vide* these Reports, II, p. 75.

4. *Experiments with* Acartomyia Zammitii.

The distribution of *Acartomyia Zammitii*, restricted as it appears to be to the Mediterranean littoral, and so corresponding to the chief incidence of Malta Fever, is held by some to give to this unique mosquito an especial claim to the carriage of the specific micro-organism of the Fever; and as moreover it is the first mosquito to appear in early summer in any considerable numbers, attention was first directed to it.

This insect, which has only comparatively recently been recognised as a distinct entity (being formerly regarded as a common Culex), is peculiar in that the early stages of its existence, from ovum to pupa, are passed in salt water. All around the coast line of the Island are rock pools above high-water mark filled with salt water, but not in direct communication with the sea, legacies of the stormy Grigali, a north-east wind which prevails from October to April. These pools remain isolated throughout the summer, and the contained salt water becomes highly concentrated from the evaporation that takes place during the hot weather.

The Acartomyia season begins in April or May, and is abruptly terminated by the first Grigali of winter. The larvæ of Acartomyia are to be found in practically all these rock pools, but are especially numerous in the half natural, half artificial salt pans in the neighbourhood of the northern and eastern coasts of the Island. It is interesting to note that a certain amount of discrimination is exercised by the female in the selection of water in which to lay her eggs. Sea water from the Mediterranean contains a larger percentage of salt

(2·72 per cent. sodium chloride) than open seas, and the average content of the more concentrated pools in which larvæ and pupæ are most abundant reaches a still higher figure (7·68 per cent.). Many attempts were made to induce the captive female to lay her eggs in fresh water or in ordinary sea water, but without success. Immediately, however, salt water of the necessary density was placed in the cage the eggs were deposited therein, even when the water was in a large glass vessel and had neither seaweed, *débris*, or empty pupa cases upon which the insect could rest during the process, and so was drowned at its termination. Again, if three vessels of water were placed simultaneously in a breeding cage containing females ready to lay their eggs, the first filled with fresh water, the second with ordinary sea water, and the third with concentrated salt water, all the eggs would be found deposited in the concentrated salt water within 24 hours, and none would be present in either of the other two vessels. The eggs are pointed ovals, averaging 0·5 mm. in length, white when first laid, but rapidly becoming brown and then black. They are laid singly or in pairs, or most commonly in rows resembling palisades, the individual eggs being arranged side by side. Under the ordinary conditions of nature, that is free exposure to air and sunlight, the eggs hatch out in from 24 to 72 hours. If transferred to fresh water immediately after being laid, the eggs do not hatch. Freshly hatched larvæ die if transferred from concentrated salt water to perfectly fresh water, but in the case of adult larvæ taken from their natural habitat and placed in tap water, development proceeds in a normal manner, but the remainder of the larval stage and the pupal stage are much prolonged. Some interesting observations on the habits of this mosquito have been recorded by Ross in a communication to the Liverpool School of Tropical Medicine.*

(a) *Duration of Life of* M. melitensis *in* Acartomyia Zammitii.

Working on the lines already indicated, several series of Acartomyia were fed on infected animals, then two or more destroyed after each of several intervals of time, the contents of the stomach of each plated out, and an enumeration made of the number of *M. melitensis* developing and the total contents of all stomachs averaged. In the later series the salivary glands were dissected carefully out and plated separately, but in no case was *M. melitensis* isolated from this situation. During the intervals between the platings the infected mosquitoes were kept alive by supplying them daily with fresh fruit, etc., or by regularly feeding them on alternate days on healthy monkeys in the attempt to transmit infection.

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* Ross, E. H., "On the Habits of the Marine Mosquito (*Acartomyia Zammitii*)," Reports of the Liverpool School of Tropical Medicine, Memoir XVIII., 1906.

Table XXIII.—*Acartomyia Zammitii* as the Host of *M. melitensis*.

No. of series.	Source of infection.	Interval between feeding and dissecting.	Number of mosquitoes dissected and plated.	Average number of *M. melitensis* per stomach.	Average number of *M. melitensis* per pair of salivary glands.
I	Guinea-pig No. 6.........	12 hours	2	116	not examined
		48 "	3	3	" "
		72 "	2	nil	" "
		96 "	2	nil	" "
II	Guinea-pig No. 80	15 minutes	2	60	not examined
		24 hours	3	17	" "
		48 "	3	386	nil
		72 "	3	6000	nil
		96 "	2	813	nil
		144 "	2	nil	nil
III	Guinea-pig No. 112......	15 minutes	3	236	not examined
		48 hours	3	nil	nil
		96 "	2	nil	nil
IV	Guinea-pig No. 113......	15 minutes	2	673	not examined
		36 hours	3	2386	nil
		48 "	2	9243	nil
		84 "	2	nil	nil
		96 "	2	nil	nil

From these preliminary investigations it will be seen that in two of the series where the original meal of blood from the infected guinea-pig contained a fairly large number of cocci, the blood present in the stomach of the host proved a suitable nidus for the ingested micrococci and that multiplication proceeded for a limited period, but by the fourth or fifth day at latest the micrococcus had disappeared from the stomach of its host, whilst examination of the salivary glands invariably failed to demonstrate its presence. It is interesting to note, and is probably explanatory of the observation, that these two series comprised insects that had been fed upon healthy monkeys in the interval between 48 and 72 hours.

By establishing plate cultivations from the black and brownish droppings of the infected mosquitoes collected from the sides and corks of the mosquito tubes, the fate of the *M. melitensis* was explained, for the excrement was found to contain the micrococcus in large numbers for the few days following the feeding, and as long as any altered blood was being voided; as soon as the excrement regained its yellowish or white colour, the *M. melitensis* ceased to be demonstrable in it. The living coccus could also be recovered from excrement voided certainly 10 days previously. The full details of four of these series of investigations are here inserted (see Table XXIII).

(b) *Virulence of* M. melitensis *after Passage through* Acartomyia Zammitii.

Having thus determined approximately the duration of the stay of *M. melitensis* in the body of the mosquito and the path by which it leaves the insect, experiments were made to determine whether the coccus undergoes any vital change as a consequence of its sojourn in an insect host. In the first place cultivations of cocci, isolated from the mosquito stomach and from the droppings respectively, were employed to inoculate healthy guinea-pigs intracerebrally.

As in each case positive results were obtained, and the animals succumbed from an acute *M. melitensis* septicæmia, further experiments were carried out in which the infected blood contained in the stomachs of individual mosquitoes was inserted under the dura mater of the guinea-pig. These experiments also gave uniformly positive results, and the inevitable conclusion is that the coccus undergoes no loss of virulence in its passage through the body of the mosquito. Details of some of these experiments are inserted in Table XXIV (p. 86).

(c) Acartomyia Zammitii *as the Infective Agent.*

Attention was now directed to the question of the transference of *M. melitensis* to the monkey *viâ* Acartomyia, and for this purpose healthy monkeys in good condition which had been under observation, housed

Table XXIV.—Virulence of *M. melitensis* after Passage through Mosquito.

Guinea-pig No.	Dose of *M. melitensis*.	Source of *M. melitensis*.	Method of inoculation.	Result.
	loop.			
I	0·1	Culture from fæces of mosquito in Series I, Table XXIII	Intracranial	Death in 30 hrs. from *M. melitensis* septicæmia.
II	0·1		,,	
III	0·1	Culture from stomach of mosquito 48 hours after feeding (Series II, Table XXIII)	,,	Death in 48 hrs. from *M. melitensis* septicæmia.
IV	Contents—blood and cocci—of stomach of Acartomyia 48 hours after feeding (*cf.* Series II, Table XXIII), mixed with 0·1 c.c. normal saline solution		Subdural	Death in less than 24 hrs. from *M. melitensis* septicæmia.

in mosquito-proof cubicles for a considerable period, and which had never shown any evidence of the presence of specific agglutinins in their blood were selected, and transferred to separate mosquito-proof cubicles on the Laboratory terrace. On the morning of the day on which it was to be subjected to the bites of the infected mosquito, the back of the thighs and legs of the experimental monkey were closely shaved, care being taken to avoid abrading the skin. The animal was then returned to its cage until the commencement of the experiment. When all was ready the animal was brought into the laboratory and stretched out face downwards on a table. The shaved areas of skin having been washed with distilled water and dried, the mosquitoes, each in its separate tube, were applied to the denuded spot and allowed to feed. This was often a tedious process and required some hours for its completion, and when using Acartomyia the feeding was often prolonged until after midnight; still, given adequate assistance in securing the monkey, as many as four of the mosquito-tubes could be manipulated by one worker.

In these experiments the process of feeding was carefully watched—to beguile the tedium of waiting as much as to ascertain the moment of repletion—and is here briefly described on account of the important bearing one feature of the process has upon the question of the infection of man through the agency of this insect. The mosquito having decided to feed, settles down at the gauze covered end of the tube, and after a few preliminary investigations with its proboscis, determines on the point of skin to be punctured. Next the head and proboscis are lowered, while the body remains more or less horizontal. Then the hind pair of legs are raised to the level of the posterior part of the abdomen, and employed in massaging the abdomen by a series of stroking movements passing from the thorax towards the anus. These movements often, but not invariably, result in the extrusion of some of the intestinal contents which, in the case of insects previously fed on mammals, is easily recognisable by reason of its colour. This small mass of excrement drops either on to the gauze covering the tubes, or where the meshes are wide, actually on to the skin of the animal upon which the mosquito is feeding. In the meantime the abdomen of the insect has become distended with blood, and as soon as the mosquito has completed its meal the proboscis is withdrawn, stroked and cleaned by the help of the anterior pair of legs, and then the insect flies up to the upper part of the tube and settles down in a resting position. This sequence of events holds good in the case of other blood-sucking species of mosquito as well as Acartomyia.

Leaving now, for a moment, the region of observed facts for a short incursion into the realms of theory, it appears highly probable that a mosquito having fed on a case of Malta Fever in the acute septicæmic stage whose blood contained large numbers of the cocci, might, 48 hours later, settle upon a healthy individual and proceed to insert its proboscis into the skin. Massaging its abdomen, the mosquito then deposits a mass of bloody excrement, enclosing the coccus in a virulent condition, upon the surface of the skin, a few millimetres distant from the puncture made by its proboscis. The bitten individual, now alive to the fact that a mosquito is feeding upon him, rouses himself, frightens off the mosquito or kills it *in situ*, and promptly proceeds to thoroughly scratch the infective excrement into the site of the minute wound and, following a successful inoculation, an attack of Malta Fever ensues. Such a sequence of events is well within the range of possibility, and yet the necessary combination of factors is sufficiently uncommon to render this method of infection as rare as the experiments upon monkeys would indicate.

To return to the actual experiments—after feeding a number of infected mosquitoes upon the monkey, the animal was returned to its cubicle and carefully observed from day to day until some four to six weeks had elapsed, when the animal was killed and carefully examined *post-mortem*.

Of the experiments carried out with the aid of *Acartomyia Zammitii*, but one monkey was infected, and in this case the infection was an extremely mild one. The history of this experiment is as follows :—

Normal Monkey No. 175 was bitten, late at night, by six Acartomyia, which had fed between 48 and 50 hours before on Guinea-pig No. 71 (which succumbed to *M. melitensis* septicæmia 10 hours after intracerebral inoculation, and the heart blood at the *post-mortem* examination was found to be absolutely teeming with the micrococci). The stomach contents of some of the mosquitoes, plated immediately after the termination of the feeding, yielded plates in which the colonies of *M. melitensis* were so numerous as to be absolutely uncountable. The bitten monkey was subsequently kept in a mosquito-proof cubicle, and every precaution which experience could suggest was taken to avoid any chance of accidental infection. The serum was tested on alternate days for the presence of *M. melitensis* agglutinins, and on the 11th day gave a positive reaction in dilution of 1 in 20. The serum value rose gradually, and on the 15th day had reacted 1 in 80, while an incomplete reaction was given with a dilution of 1 in 100. On the 21st day no reaction could be obtained, even with 1 in 10 dilutions, therefore the animal was killed. During the period of three weeks, in which the monkey was under observation, the temperature chart, which is here inserted, gave no indication whatever of a successful subcutaneous inoculation.

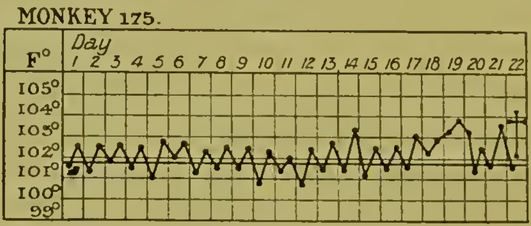

Chart 10.

At the *post-mortem* examination the spleen was found to be slightly enlarged, and all the superficial glands were markedly enlarged and congested.

In none of the plate cultivations prepared from heart blood, spleen pulp, and axillary and inguinal glands could *M. melitensis* be detected, but in one of the three tube cultures prepared from spleen pulp two colonies appeared of a minute coccus, which when tested against specific serum became agglutinated, although in a somewhat atypical manner. Subcultivations were prepared from these, and further investigation proved that one of the two colonies was composed of genuine *M. melitensis*.

In none of the other monkeys did the serum at any time during the period of observation contain specific agglutinins, nor could the presence of *M. melitensis* be detected in the blood. Finally, at the *post-mortem* examination *M. melitensis* was never recovered from any of the other organs or tissues.

Before tabulating the results of those of the Acartomyia experiments in which the value of each factor was accurately known, it may be useful to recapitulate the exact method of procedure—a reiteration which the importance of the subject alone condones :—

(*a*) First a healthy guinea-pig was inoculated intracerebrally with a sufficient dose of highly-virulent culture of *M. melitensis* to produce such an acute infection that in the course of a few hours large numbers of the cocci would be present in the peripheral blood.

(*b*) Next, when the temperature of the inoculated guinea-pig had reached 105° F. or 106° F. (a point which previous experience had shown corresponded to rapid multiplication of cocci in the blood), the first feeding of the mosquitoes was commenced.

(*c*) Several female mosquitoes—the number varying with the experiment from 20 to 70—each enclosed in a separate feeding tube, were applied one by one to a bare patch of skin between the infected guinea-pig's shoulders and encouraged to bite. As soon as one mosquito was seen to be full of blood, and to have settled to repose in another part of the tube, the tube was taken away, marked with its serial number, the number of the guinea-pig, and the date and time of feeding, and replaced by the next tube containing a fasting mosquito.

(*d*) During the progress of the feeding experiment, or immediately after its termination, some of the mosquitoes (the number varying in each experiment with the number of mosquitoes that had fed and were available) were killed with ether vapour, and the contents of the stomach plated out to determine the average number of cocci ingested with the blood of the guinea-pig. The feeding tubes containing the remainder of what may now, for purpose of reference, be termed "infected mosquitoes" were placed in test-tube racks and put on one side.

(*e*) Forty-eight hours after the primary feeding of the mosquito a healthy monkey was selected, stretched face downwards on a table, and the back of the fleshy part of each thigh and leg shaved, great care being taken to avoid cutting or grazing (this process was often carried out some hours earlier in the day). The shaved areas were washed with distilled water and partly dried with sterile wool.

(*f*) Each of the infected mosquitoes was now applied in turn to the shaved area, and allowed to bite the monkey and fill itself with blood. Some of course refused; these were put aside for feeding at 72 hours.

(*g*) At the termination of the feeding on the monkey two or three of the infected mosquitoes that had filled themselves were plated out to determine whether the contents of their stomachs still contained the *M. melitensis*, and to prove beyond question that the mosquitoes that bit the experimental animal actually contained infective material at the moment they were engaged in feeding on the monkey.

(*h*) At 72 hours after the primary feeding such infected mosquitoes as had refused to bite the first monkey (at 48 hours) and were still living, were applied to a second healthy monkey, and if they bit and filled themselves with blood the same routine was repeated.

Table XXV.—Transmission of *M. melitensis viâ* Acartomyia.

Infective agents.					Experimental animals.			
Source of infection.	Average number of *M. melitensis* per control mosquitoes.		Interval between primary feeding and biting monkey.	Number of mosquitoes employed in experiment.	No.	Date of appearance of serum reaction.	Duration of experiment in days.	*Post-mortem* findings.
	Immediately after feeding.	At time of biting monkey.	hours.					
Guinea-pig No. 90...	2426	2070	48	12	Monkey No. 177	—	34	nil
Guinea-pig No. 112...	236	nil	48	8	Monkey No. 186	—	28	nil
Guinea-pig No. 113...	673	9243		8				
Guinea-pig No. 71...	∞	∞	48	5	Monkey No. 175	11th day	21	1 colony of *M. melitensis* in one of the many cultures from spleen: other organs nil
Guinea-pig No. 72...	∞	∞	75	1	Monkey No. 176	—	43	nil
Guinea-pig No. 90...	2426	103	96	7	Monkey No. 178	—	32	nil
Guinea-pig No. 112...	236	nil	96	9	Monkey No. 187	—	26	nil
Guinea-pig No. 113...	673	nil						
Guinea-pig No. 90...	2426	nil	144	2	Monkey No. 179	—	24	nil

∞ = innumerable.

(*i*) At 96 hours after the primary feeding the infected mosquitoes (diminished in number as a result of death from natural causes or destruction for plating purposes) were applied to a third, and so on as long as any of the mosquitoes remained alive.

(*j*) The monkeys after being bitten were placed in mosquito-proof cubicles, and observed from day to day as to temperature and general condition, blood reactions, etc., until the termination of the experiment, when a careful *post-mortem* examination was carried out.

It was usual not possible to carry the above scheme beyond the 96 hours, owing to the death of the infected mosquitoes, as will be seen in Table XXV.

5. *Experiments with* Stegomyia fasciata.

Having dealt at some length with those mosquito experiments in which *Acartomyia Zammitii* was the species employed, those in which *Stegomyia fasciata* was employed as the infective agent may be briefly dismissed, for the technique was identical in both series of experiments.

(a) *Duration of Life of* M. melitensis *in* Stegomyia fasciata.—Experiments were made to determine the duration of life of *M. melitensis* in the stomach and intestines of Stegomyia in the manner already described (*vide* pp. 81 and 82), and the results obtained were very similar, for it was found that the micrococcus could rarely be recovered from this mosquito beyond four days after the primary feeding on the infected guinea-pig. The results of a few representative experiments are given below in Table XXVI:—

Table XXVI.—*Stegomyia fasciata* as the Host of *M. melitensis.*

No. of series.	Source of infection.	Interval between feeding and dissection.	Number of mosquitoes dissected and plated.	Average number of *M. melitensis* per stomach.
I	Guinea-pig No. 140	24 hours	2	∞
		48 ,,	2	6177
		72 ,,	3	*
		84 ,,	2	nil
II	Guinea-pig No. 150	30 minutes	3	∞
		48 hours	3	44
		96 ,,	2	nil
III	Guinea-pig No. 151	2 hours	1	∞
		24 ,,	2	44
		48 ,,	2	5
		72 ,,	2	*
		96 ,,	3	nil

∞ = innumerable.

* *M. melitensis* present, but plate unworkable owing to contamination with saprophytes probably derived from exterior surface of mosquito's abdomen.

(b) Stegomyia fasciata *as the Infective Agent.*—The experiments in which the attempt was made to transmit *M. melitensis* from the infected guinea-pig to the healthy monkey *viâ Stegomyia fasciata*, were planned and carried out in a manner identical with those detailed in connection with *Acartomyia Zammitii* (*vide* p. 86), consequently the technique need not again be described. The only point to be noted is that, as the solitary infection produced when Acartomyia was employed as the infective agent ensued when the mosquitoes bit a healthy monkey 48 hours after the primary feeding on the infected guinea-pig, the majority of the Stegomyia experiments were performed at that interval.

The possibility that infection might be produced as the result of puncturing the skin of the healthy subject by the soiled proboscis of a mosquito that had partly filled itself from an infected patient, was tested experimentally in one instance only, but in that single instance—as events subsequently proved—the conditions were exceptionally favourable to the transmission of the specific virus. The details of this experiment were as follows:—

One female Stegomyia, in its feeding tube, was applied to the back of an infected guinea-pig (prepared for the experiment by intracranial inoculation of *M. melitensis* 8½ hours previously) and allowed to settle down, insert its proboscis into the skin, and commence to feed. The instant that blood could be distinguished entering its abdomen, the feeding tube was removed and applied to the shaven leg—immediately over the external saphenous vein of Monkey No. 236, and the mosquito allowed to bite and complete its meal. Within half an hour of its removal from the monkey, the contents of this mosquito's stomach were plated out, but none of the plates that were prepared showed a single colony of *M. melitensis.*

Immediately the first mosquito had completed its meal, a second Stegomyia was applied to the guinea-pig and allowed to bite and about half fill its abdomen with blood. It was then transferred to the same monkey and applied, again over the external saphenous vein, at a point a centimetre or so above the first puncture. The mosquito settled down at once, inserted its proboscis, and proceeded to fill itself up with the monkey's blood. A short time after the mosquito had completed its meal, it was killed and the contents of its stomach plated out. Subsequent enumeration of the colonies of *M. melitensis* that developed on the plates showed that the stomach of this mosquito contained about 5000 cocci.

Following the feeding of the second mosquito, a third Stegomyia was half fed on the same infected guinea-pig and then transferred to the monkey and allowed to bite over the vein, just between the first and second mosquito bites, and complete its meal. All the plate cultivations prepared from the stomach contents of this third mosquito

Table XXVII.—Transmission of *M. melitensis viâ* Stegomyia.

Infective agents.					Experimental animals.			
Source of infection.	Average number of *M. melitensis* per mosquito.		Interval between primary feeding and biting monkey.	Number of mosquitoes employed in experiment.	No.	Date of appearance of serum reaction.	Duration of experiment in days.	*Post-mortem* findings.
	Immediately after feeding.	At time of biting monkey.						
Guinea-pig No. 140.........	∞	6,177	48	5	Monkey No. 207	—	44	nil
Guinea-pig No. 181.........	?	350	48	5	Monkey No. 228	—	37	nil
Guinea-pig No. 151.........	∞	44	48	18	Monkey No. 230	—	40	nil
Guinea-pig No. 150.........	∞	10,000	48	12	Monkey No. 232	—	69	nil
Guinea-pig No. 150.........	∞	nil	96	8	Monkey No. 231	—	38	nil
Guinea-pig No. 140.........	∞	4,855*	15 secs.	3*	Monkey No. 236	—	46	nil
Guinea-pig No. 170.........	22	320	48	1	Monkey No. 238	—	37	nil

∞ = innumerable.

* All three mosquitoes were killed and examined ; *M. melitensis* was absent from the stomach of the first, present to the number of 4855 in the second, and in innumerable numbers in the third.

yielded pure growth of *M. melitensis*, in which the colonies were so numerous as to be absolutely uncountable.

The monkey that had been bitten by these three mosquitoes, however, remained perfectly healthy, specific agglutinins remained absent from its blood, and, when killed and examined *post mortem* 46 days later, showed no signs of infection with *M. melitensis*.

Two of the three mosquitoes, therefore, that first sucked blood from an infected guinea-pig and then finished their meal upon blood abstracted from a healthy monkey were conclusively shown to have taken up with the guinea-pig's blood a very large number of the micrococcus and, one would infer, had had ample opportunity of soiling the proboscis in each case, yet the monkey remained uninfected.

Details of the experiments in which the various data were accurately ascertained are tabulated below; other similar experiments were performed, but, owing to failure of plate cultures and other causes, there was no evidence as to the numbers, or, indeed, the presence of *M. melitensis*, in the interior of the mosquitoes used for biting. Such experiments being worthless, are not included in the results presented in Table XXVII.

The net result of the mosquito experiments may now be summarised very briefly. Out of 14 experimental monkeys bitten under conditions which have been accurately recorded (and disregarding a further six, where the necessary data are missing in whole or in part, but in none of which did infection occur), one monkey alone has given evidence of actual infection. In this one positive case, the *M. melitensis* contained in the stomach of the mosquito at the time it bit the monkey—48 hours after the primary feeding on the infected guinea-pig—were innumerable in quantity, and the inference is justifiable that, if excrement was dropped on the skin while feeding on the monkey, numerous living and virulent *M. melitensis* must have been present therein. Consequently, after removal from the operating table there was nothing to prevent this monkey (or, indeed, any of the experimental monkeys) scratching the infective material into the puncture, and this is quite possibly a case in which some such variety of infection occurred.

6. *Experiments with other Species of Mosquitoes.*

(a) *Theobaldia spathipalpis.*—The opinion has been freely expressed in some quarters that the members of this species are fruit feeders only, and never suck blood. This statement, however, does not hold good for Malta, as, during the summers of 1905 and 1906, whilst examining specimens of mosquitoes captured in various parts of the Island, *Theobaldia* was sometimes noted gorged with blood. One of the members of the Commission received several specimens in the course of the winter of 1905—1906, which had been "caught feeding" on the human subject, one of which had so recently fed that the blood cells were unaltered and could readily be demonstrated microscopically. This particular specimen was submitted to Mr. E. E. Austen for confirmation of identity, and his reply is here inserted:—

Copy of letter from Mr. E. E. Austen.

BRITISH MUSEUM (Nat. Hist.),
CROMWELL ROAD,
LONDON, S.W.,
10*th January*, 1906.

Dear Sir,

I am very sorry that your letter of December 9th, enclosing a specimen of *Theobaldia* (*Culex*) *spathipalpis*, has remained so long unacknowledged. The letter arrived here while I was absent on a month's vacation, and since I have only just returned to duty, it was consequently impossible for me to reply sooner. Please forgive the delay.

Your identification of the species is perfectly correct, and the notes in your letter on its occurrence and blood-sucking habits in Malta are very interesting. I see you say that "it is described by Giles and Theobald as non-blood-sucking," but if you look at Theobald's monograph, vol. i, p. 341, you will see that the species is stated (apparently on the authority of Major Birt) to be "troublesome" in Gibraltar; it would appear, however, from the following page that Ficalni believes the species to be a vegetable feeder, and Grabham, writing with reference to Madeira in Theobald's monograph, vol. iii, p. 155, says "the people told me they had never observed this form attacking man or animals. They fed eagerly on banana slices, but never attempted to bite my hands."

It would be remarkable if *Theobaldia spathipalpis* should prove to be exclusively vegetarian in some localities, but a blood-sucker in others.

Believe me,
Yours very truly,
(Signed) E. E. AUSTEN.

Captain J. Crawford Kennedy,
R.A.M.C.

In view of these observations, numerous attempts were made to induce female *Theobaldia*, some captured and others bred in captivity, to bite infected guinea-pigs, but with negative results. Neither could they be persuaded to bite either monkeys or man.

(b) *Culex fatigans* and *Culex pipiens*.—For all practical purposes these two species are identical, and in breeding out in the Laboratory no attempt was made to differentiate between them.

Very few experiments were made with these mosquitoes, as it was a matter of the greatest difficulty to induce them to feed on the guinea-pig in the first instance, or on the monkey in the second. Further, whilst on the one hand no infection resulted, on the other no proof of the infective character of the mosquito was forthcoming, as plate cultivations of the stomach contents were omitted—consequently the experiments referred to were worthless.

(c) *Anopheles maculipennis.*—No experiments were made with this mosquito, as its only breeding place is situated in a small, almost uninhabited valley in the centre of the island, and the imagines are not met with elsewhere than in its immediate vicinity. It is therefore obvious that this mosquito can have next to nothing to do with the dissemination of *M. melitensis.*

7. *Biting Flies*—Stomoxys calcitrans.

Although the experiments of Edmond and Etienne Sergent* have shown that *Stomoxys* does not usually act as the infective agent concerned in the transmission of the trypanosome of North African camel disease, the fact that one successful infection in the rat was produced through the medium of this fly, encouraged the hope that in the case of such a minute coccus as *M. melitensis* success might attend experiments in the direction of transmission of the micrococcus to the goat by means of this biting fly.

(a) *Duration of Life of* M. melitensis *in* Stomoxys calcitrans.—As in the case of the mosquito, an inquiry was first made into the ability of *Stomoxys* to act as the host to *M. melitensis*, and the experiments were conducted on identical lines. Abundant supplies of *Stomoxys* were readily obtained from stables, so that it was unnecessary to breed them. When captured they were placed in a fair-sized gauze-covered cage, brought up to the Laboratory, and, as the insects were for the most part caught whilst feeding on the horses or mules, they were usually kept for a day before using to enable them to dispose of the blood with which they were filled. They were then transferred singly to feeding-tubes similar to those used for mosquitoes.

A guinea-pig was then infected, and when its blood was crowded with the micrococci, flies, each enclosed in a feeding-tube, were applied in turn to an area of skin denuded of hair and allowed to fill themselves with blood.

It was soon found that the wastage from death was excessive unless these insects were fed every 24 hours; this necessitated feeding the balance left over after every plating period on a healthy guinea-pig every day, but even this plan did not entirely prevent the heavy mortality.

At varying intervals several flies were killed by ether vapour, dissected, the contents of the stomach and intestines plated out, and the resulting colonies of *M. melitensis* enumerated.

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* 'Annales de l'Institut Pasteur,' vol. 19, 1905, pp. 16 *et seq.*

Plate cultivations were also established from the droppings, and it was found that, as in the mosquito, the excrement contained numerous living *M. melitensis* for many hours after feeding upon the infected guinea-pig.

In the following table the results of a number of these experiments are arranged, and from that it will be seen that although the micrococcus has sometimes disappeared entirely from the alimentary tract within a couple of days, it may persist even up to five days.

Table XXVIII.—*Stomoxys calcitrans* as the Host of *M. melitensis.*

No. of series.	Source of infection.	Interval between primary feeding and dissection.	Number of Stomoxys dissected and plated.	Average number of *M. melitensis* per stomach and intestines.
I	Guinea-pig No. 72	15 hours	1	197
		24 ,,	1	nil
		36 ,,	1	19
		60 ,,	6	136
II	Guinea-pig No. 82	15 minutes	2	832
		24 hours	1	5
		48 ,,	1	nil
		72 ,,	1	nil
		96 ,,	1	nil
III	Guinea-pig No. 111	15 minutes	2	347
		18 hours	2	17
		40 ,,	2	5
		97 ,,	2	2
IV	Guinea-pig No. 122	15 minutes	5	2,248
		24 hours	3	nil
		48 ,,	3	nil
		86 ,,	3	nil
		100 ,,	3	400
V	Guinea-pig No. 150	2 hours	3	912
		90 ,,	2	nil
VI	Guinea-pig No. 162	1 hour	2	3300
		48 hours	2	nil
		120 ,,	6	50,352

(b) Stomoxys calcitrans *as the Infective Agent.*—The experiments in which infected Stomoxys were allowed to bite healthy animals were conducted on similar lines to the corresponding experiments with mosquitoes. Two points of difference only need noting.

In the first place, the kid which previous experiments had shown (*vide*

Table XXIX.—Transmission of *M. melitensis viâ* Stomoxys.

Infective agents.				Experimental animals.			
Source of infection.	Average number of *M. melitensis* per control *Stomoxys* immediately after primary feeding.	Interval between primary feeding and biting the kid.	Number of *Stomoxys* employed in the experiment.	Animal and No.	Date of appearance of serum reaction.	Duration of experiment in days.	*Post-mortem* findings.
Guinea-pig No. 72...	197	20	4	Kid No. 1 ...	—	70	nil
Guinea-pig No. 122...	2,000	20	9	Kid No. 2 ...	—	28	nil
Guinea-pig No. 122...	2,000	44	6	Kid No. 3 ...	—	27	nil
Guinea-pig No. 122...	2,000	112	3	Kid No. 6 ...	—	35	nil
Guinea-pig No. 162...	3,000	24	12	Kid No. 7 ...	—	9	nil
Guinea-pig No. 162...	3,000	48	6	Kid No. 8 ...	—	42	nil
Guinea-pig No. 162...	50,352	96 102 120	6 5 6	Kid No. 15 ...	—	40	nil

pp. 30 and 34) was extremely susceptible to subcutaneous and cutaneous inoculation, replaced the monkey as the experimental animal; and in the second place the infected *Stomoxys* were fed upon the healthy guinea-pig in the intervals between feeding on the kid. The results may be very briefly stated. In all, seven experiments were carried out, and in no case did infection of the kid with *M. melitensis* ensue, although in one of the experiments a kid was bitten by three separate batches of six, five, and six *Stomoxys* respectively during a period of 24 hours, at intervals of 96, 102, and 120 hours after the primary feeding on the infected guinea-pig. The details of these experiments are arranged in tabular form under the same headings as those used for the corresponding mosquito experiments on pp. 90 and 93.

8. *Fleas and Bugs.*

The experiments carried out with blood-sucking Hemiptera and Aphaniptera were few in number and of very questionable value, even when considered in relation to the negative evidence they produced, and may be very briefly dismissed.

Hemiptera—*Cimex lectularia.*—In view of the suggested association between certain cases of Malta Fever and venereal disease referred to by Kennedy* and the possibility of the infective virus being conveyed by domestic vermin, some 50 ordinary bed bugs were collected from low class brothels in the vicinity of the Camerata, Valletta, conveyed to the Laboratory and there killed and dissected. The stomach contents of each were then plated out in turn, but *M. melitensis* was never detected in, or isolated from, this situation. These were the only observations made with this parasite.

Aphaniptera (a) *Pulex irritans.*—No observations were made upon the human flea.

(b) *Pulex serraticeps.*—Dog fleas to the number of six were collected from a dog (which had been experimentally infected with *M. melitensis* by subcutaneous inoculation), killed, dissected, and plated, but no evidence could be obtained to show that they harboured the micrococcus.

(c) *Pulex scuirorum.*—Again 14 rat fleas were obtained from various rats which had been experimentally infected with *M. melitensis*, and in whose blood *M. melitensis* was swarming. After being similarly dissected the plate cultivations established from the stomach contents likewise failed in every case to show the presence of the coccus.

Two experiments were made in an endeavour to transmit *M. melitensis* from infected to healthy dogs through the agency of the flea:—

1. A healthy black terrier dog was inoculated subcutaneously

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* Kennedy, 'Journ. Royal Army Med. Corps.,' vol. 4, 1905, p. 634.

behind the shoulder with emulsion containing the growth from three agar tubes of *M. melitensis*. Three weeks later the animal, which had in the interval shown definite signs of infection, was killed with chloroform vapour and the dead body placed close to, but not in contact with a bitch of the same variety for a period of 24 hours, in the hope that infected fleas would pass from the dead body as it cooled to the warm body of the living animal and so transmit the infection. *M. melitensis*, however, was only isolated *post mortem* from the inguinal and mesenteric glands of the terrier dog—not from the blood. The bitch never showed any sign of infection during an observation period of two weeks, and at the end of this time the second experiment was proceeded with.

2. Another black terrier dog which had been inoculated with a similar dose of *M. melitensis* six weeks previously, and like the first dog had given definite evidence of infection, was killed and placed alongside the same terrier bitch that had been utilised for the first experiment, for about 14 hours—precautions being taken to prevent contact of the healthy bitch with either carcase or excrement of the infected animal. This second dog when examined *post mortem* gave evidence of the presence of *M. melitensis* in spleen, inguinal and axillary glands, but the coccus was again absent from the blood.

The bitch never showed any signs of infection.

These two experiments are of course valueless from the scientific point of view, as the evidence available points to the absence of micrococci from the blood of the infected animal at the time of death, even supposing the dog fleas travelled from the carcase to the body of the living bitch.

Some further experiments were then made on somewhat similar lines with the rat flea. The rat had always been considered by the early workers with *M. melitensis* as insusceptible to infection with this organism, but it was observed by one of the members of the Commission that the rat, after intracranial inoculation, dies from acute septicæmia in about 24 hours, the micrococcus being present in the blood in enormous numbers for at least the last 12 hours of life; while after a few intracranial passages the coccus becomes exalted in virulence for this particular animal and readily produces an acute septicæmia after either intraperitoneal or subcutaneous inoculation. Consequently in investigating the probability of the transmission of *M. melitensis* from the infected to the healthy rat, these highly virulent cultures were utilised.

In arranging these experiments, long rat cages were selected and divided into three compartments, a fair-sized one at either end, and a small narrow one in the centre, by means of two wire-netting partitions placed about 6 cm. apart. The meshes of the wire netting forming the partitions were about 0·5 cm. in diameter.

A couple of infected white rats were placed in one end compartment and two healthy rats in the other; all four rats were infested with large and vigorous fleas. The cage was then placed in a large rectangular zinc tray filled with lysol solution almost to the level of the floor of the cage. In the first experiment the infected rats died within 18 hours, and on the morning following the commencement of the experiment it was found that the other two rats had partly broken down the partitions and were occupying the same compartment as the dead rats.

The infected animals examined *post mortem* were noted to be quite free from fleas. The blood of each was crowded with the micrococci, as were spleen, kidneys, and all other organs.

The healthy rats died six hours after the infected animals, and at the *post-mortem* examination, which was conducted at once, no fleas could be found on the carcases, although shortly before death the animals were simply swarming with them. Many dead fleas were, however, found in the lysol moat surrounding the cage. From the *post-mortem* examination no evidence of infection could be obtained, as *M. melitensis* could not be detected in, or isolated from any of the tissues or organs.

This experiment was repeated a fortnight later in precisely the same manner.

Again the infected rats soon succumbed—one within nine hours, its companion within 22 hours after inoculation. No fleas could be detected on the carcases and no dead fleas were present in the lysol. *Post-mortem* blood and organs were crowded with micrococci.

The healthy rats were killed with chloroform seven days later and examined at once. Specific agglutinins were absent from the blood of each. Plate cultures from the blood and various organs failed to show any colonies of *M. melitensis*, neither could the micrococcus be detected in the bodies of the fleas caught on the bodies of these rats.

In both these experiments the *M. melitensis* was undoubtedly present in the infected rats, and the fleas upon them in all probability absorbed the cocci with the blood of their hosts. Further, it is a matter of common knowledge that fleas forsake the bodies of dead or dying rats for healthy living members of the same species, and in these experiments should have had ample opportunity of biting the healthy rats in the further compartment. Yet in neither of the four rats exposed to bites from presumably infected fleas did infection with *M. melitensis* ensue and the results were absolutely negative.

V.—NATURALLY ACQUIRED INFECTION (*M. melitensis*) IN VARIOUS ANIMALS.

During 1905 numerous observations were carried out upon various animals during life to determine whether infection with *M. melitensis* occurred naturally. These observations were usually limited to an examination of the blood for *M. melitensis* agglutinins, but were followed in a few cases by *post-mortem* examination. As a result of these examinations, it was clearly shown that cows and dogs living in the Island acquired naturally an infection with *M. melitensis*, while from the presence of positive serum reactions in low dilutions, it was suggested that mules were also susceptible to the pathogenic action of the coccus.

Similar observations were continued during the summer of 1906, as many various species of animals being examined as opportunity permitted, and the results noted are here recorded.

1. *Rats.**—The close association of epizootic plague in the rat and epidemic plague in man suggested the possibility of a similar association between the rat and Malta Fever, but inquiry amongst residents in the Island failed to support this view.

Arrangements were then made for the supply of sewer rats from the dockyard and from the Valletta sewers for examination in the Laboratory. Owing, however, to the frequent and prolonged absence of the Fleet from Malta, the number of rats obtainable from the dockyard was small; it also proved a matter of greater difficulty to obtain the rodents from the Valletta sewers, even a capitation fee of 3*d.* per head failing to secure the necessary material, so that a total of 84 rats examined during the months of May, June, and August is all that could be obtained. During May and June, the rats on arrival at the Laboratory were sorted out into cages and numbered, and blood collected from each to afford serum for the agglutination reaction, and also for planting out for the recovery of *M. melitensis*, and it was intended that a complete *post-mortem* examination should be made only when specific agglutinins were detected in the blood.

Table XXX.—Examination of Sewer Rats.

Month, 1906.	Number of rats examined.	Serum reaction.	*M. melitensis* in	
			Blood	Other organs.
May…………	72	Absent	Absent	Not examined.
June ………	6	”	”	”
July ………	0	—	—	—
August ……	8	Absent	Absent	Absent

In July no sewer rats at all could be obtained, but during that month experiments were carried out which showed that while the rat was susceptible to infection with the micrococcus, agglutinins were not usually formed during the course of the disease, and consequently a complete *post-mortem* was performed on the eight rats that were available during August.

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* Compare these Reports, V, p. 40.

The results of these examinations were completely negative. None of the specimens of rat blood that were examined yielded an agglutination reaction even in low dilutions, and *M. melitensis* was never recovered from blood or organs.

Several dozen white rats, bred on the Island and purchased for experimental work, were also tested before use as to the presence of the serum reaction to *M. melitensis*, likewise with negative results.

2. *Dogs.**—Forty-six stray dogs were examined between the beginning of March and the end of August for the presence of *M. melitensis*, and of these three yielded a positive reaction—one in dilution of 1 in 10, one in dilution of 1 in 20, and a third in all dilutions up to 1 in 100. Although a reaction obtained in a dilution of 1 in 20 is by no means absolute or even satisfactory evidence of infection, all three of these animals were killed and examined *post mortem*, but *M. melitensis* was never recovered from any of the organs or tissues.

Table XXXI.—Examination of Stray Dogs.

Month.	Number of dogs examined.	Serum reaction.	*M. melitensis* in	
			Blood.	Other tissues and organs.
March	10	1 × 1 in 10	Not examined	Not examined.
April	13	Absent	,,	,,
May	0	—	,,	,,
June	0	—	,,	,,
July	0	—	,,	,,
August	23	1 × 1 in 20	Absent	Absent
		1 × 1 in 100	,,	,,

3. *Guinea-pigs.*—The numerous guinea-pigs employed in the experimental work were all bred on the Island, and as a preliminary precaution the blood was carefully examined in many different dilutions with reference to the presence of specific agglutinins. These substances, however, were invariably absent from the blood.

4. *Mules.**—Some mules were imported into Malta from Sicily at the beginning of 1906. Steps were taken to examine their blood as soon as possible after arrival. As a result of this examination the blood from each of these mules yielded a partial or incomplete reaction with serum in low dilution (1 in 10), but no reaction whatever could be obtained in higher dilutions.

Six months later the blood from these same nine mules was again

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* Compare these Reports, III, p. 85 *et seq.*

Table XXXII.—Examination of Mules.

No. of mule.	Date of arrival in Malta.	Date of first examination.	Agglutination value of mule's serum.	Nature of sickness, if any, recorded between February and August, 1906.	Date of second examination.	Agglutination value of mule's serum.
48,396	19/2/06	15/3/06	1 : 10 ±	Girth gall	28/8/06	1 : 200 +
48,397	19/2/06	15/3/06	1 : 10 ±	Nil	28/8/06	1 : 10 +
48,398	19/2/06	15/3/06	1 : 10 ±	Nil	28/8/06	nil
48,399	19/2/06	15/3/06	1 : 10 ±	Girth gall : swollen sheath	28/8/06	1 : 10 ±
48,400	19/2/06	15/3/06	1 : 10 ±	Castration	28/8/06	nil
48,401	19/2/06	15/3/06	1 : 10 ±	Set fast	28/8/06	1 : 500 +
48,402	19/2/06	15/3/06	1 : 10 ±	Breast harness gall	28/8/06	1 : 10 ±
48,403	19/2/06	15/3/06	1 : 10 ±	Sore breast : lame	28/8/06	1 : 200 ±
48,404	19/2/06	15/3/06	1 : 10 ±	Nil	28/8/06	1 : 500 ±

examined, with the result that one reacted well and one incompletely in dilution of 1 in 500; two others reacted, one completely and one incompletely in dilutions of 1 in 200. The serum reactions of the remaining mules remained practically unaltered.

Judging these results from the alteration in their serum values, it is practically safe to assume that two of the mules (Nos. 48,401 and 48,404) had acquired *M. melitensis* infection during the course of their first summer in Malta, and probably Nos. 48,396 and 48,403 had also become infected. The absence of any serious sickness in these animals needs no comment in view of the lack of constitutional symptoms in the infected goats. Unfortunately, *post-mortem* examination of these animals was not possible. The results of these examinations are given in Table XXXII.

5. *Horses.*—In order to obtain some idea as to the prevalence of *M. melitensis* infection in the horse—as judged by the serum reaction standard—samples of blood from 100 horses attached to the Mounted Infantry camp at Ghain Tuffeiha were examined.

The results obtained were as follows :—

Negative reaction	54
Incomplete reaction in 1 in 10 dilution	14
Complete ,, ,, ,,	11
Incomplete ,, 1 in 20 ,,	11
Complete ,, ,, ,,	7
Incomplete ,, 1 in 50 ,,	2
Complete ,, ,, ,,	1
	100

It will thus be seen that the agglutination reaction was obtained in varying dilutions in 46, the test proving absolutely negative in 54. In 14 of those animals yielding a reaction of some sort, the result of the test was "incomplete," clumping with 1 in 10 dilutions, and as such a reaction is valueless as evidence of infection, these animals may be disregarded. Again, neither a complete reaction in a dilution of 1 in 10, nor an incomplete reaction in dilution of 1 in 20 can be regarded as incontrovertible evidence, and at any rate, in the case of the human subject, neither would be accepted as deciding a question of diagnosis. Eliminating such animals as did not give a positive reaction in at least as high a dilution as 1 in 20—and even this standard is open to question—10 animals are left which show signs of infection by *M. melitensis*, three, indeed, in a marked manner. Unfortunately, no further examination of these infected animals was possible.

One of the animals (No. 95) was subsequently shot, and examined *post mortem*. During life the titre of its serum, when tested against

Table XXXIII.—Examination of Horses.

Serial No.	Service in Malta.	Agglutination value of serum.	Previous medical history.	Present condition.
6	2 years	1 : 10 ±	Nil	Good
25	5 ,,		One attack colic	Good
27	5 ,,		Nil	Good
42	5 ,,		Two attacks colic: one attack catarrh	Good
98	5 ,,		Nil	Good
113	—		—	—
117	5 years		One attack catarrh	Good
119	5 ,,		Nil	Good
120	5 ,,		Nil	Good
125	2 ,,		Nil	Fair
126	2 ,,		Nil	Good
138	1 year		Nil	Good
140	4 years		Two attacks colic: one attack horse-pox	Good
145	1 year		One attack diabetes	Poor
2	6 years	1 : 10 +	One attack stomatitis	Fair
23	6 ,,		Nil	Good
30	5 ,,		Two attacks colic	Fair
43	2 ,,		Nil	Good
72	6 ,,		One attack catarrh	Fair
75	6 ,,		Nil	Good
95	6 ,,		Nil	Good
103	5 months		Nil	Fair
114	6 years		Nil	Good
130	5 months		Nil	Good
137	5 ,,		Nil	Fair
9	6 years	1 : 20 ±	Nil	Good
18	6 ,,		One attack catarrh	Fair
22	6 ,,		Nil	Good
56	6 ,,		Nil	Good
58	5 ,,		Nil	Good
59	5 ,,		Nil	Good
61	5 ,,		One attack strangles	Good
70	5 ,,		Nil	Good
82	6 ,,		Nil	Good
91	5 ,,		One attack catarrh	Good
127	6 ,,		One attack catarrh	Fair
37	6 ,,	1 : 20 +	One attack pink eye	Good
38	2 ,,		Nil	Good
66	6 ,,		Nil	Good
71	6 ,,		Nil	Good
115	6 ,,		Two attacks catarrh	Good
129	6 ,,		Two attacks debility	Fair
143	1 year		Nil	Fair
64	2 years	1 : 50 ±	Nil	Good
74	6 ,,		Nil	Good
94	6 ,,	1 : 50 +	Nil	Good

M. melitensis, was 1 in 10. The micrococcus could not, however, be detected in either blood, spleen, or inguinal or mesenteric glands. Three other horses (Nos. 11, 35, and 52), whose blood during life showed no signs of the presence of agglutinins, were examined *post mortem*, but with completely negative results so far as related to the presence of *M. melitensis*.

Details of the animals, relating to the length of service in Malta with the Mounted Infantry, their present condition, and previous medical history, were kindly supplied by the commanding officer, Captain Salmon, and such of those details as refer to horses that gave a serum reaction are given in Table XXXIII.

VI.—*M. melitensis* INFECTION BY MEANS OF PERSONAL CONTACT.

The incidence of Malta Fever upon cases admitted to naval and military hospitals for venereal disease, although probably due to infection by means of the goats' milk that forms the staple article of diet of the men during the first days of treatment, at once raises the suggestion that simultaneous infection might occur during sexual connection through minute lesions of the mucous membrane of the glands or prepuce, especially when considered in conjunction with the existence of *M. melitensis* in the urine of so-called ambulatory cases of the disease. In these latter cases febrile disturbance is extremely slight or entirely absent, as indeed are clinical symptoms of any kind, although at the same time the patient, male or female, may be passing *M. melitensis* in thousands per cubic centimetre in the urine.

Writing in the 'Journal of the Royal Army Medical Corps,'* Kennedy stated that of the cases which developed Malta Fever after admission to the military hospital for some other disease, by far the larger proportion were venereal cases. The experience of the authorities at the Royal Naval Hospital appears to be very similar.

Following up this line of enquiry, the before-mentioned observer collected in 1905, and followed out, a series of 124 cases, 20 of which had been under treatment for venereal disease during some portion of the couple of months preceding the diagnosis of Mediterranean Fever. Again, of these 20 cases the probability of infection contracted within the hospital could be excluded with tolerable accuracy in 12, viz., 6 cases of gonorrhœa, 1 of soft sore, and 5 of syphilis.

Under these circumstances an enquiry was instituted into the medical history of the common prostitutes of Malta. The investigation was much facilitated owing to the fact that the bulk of these women are Italians or Sicilians and, therefore, coming under the law with regard to aliens, are registered by the police, their addresses are known, and a

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* Kennedy, J. C., "Malta Fever in the Military Hospital, Valletta, Malta, during the years 1897—1904," 'Journ. R.A.M.C.,' vol. 4, 1905, pp. 634—646.

Table XXXIV.—Positive Results of Agglutination Test.

No.	Initials.	Duration of residence in Malta.	History of attack of Malta Fever.	Serum value.					
				1 : 5.	1 : 10.	1 : 20.	1 : 50.	1 : 100.	1 : 200.
94	N. A.	9 months	Negative	+	±	−	−	−	−
95	C. A.	20 ,,	,,	+	±	−	−	−	−
96	R. A.	1 year	,,	+	±	−	−	−	−
97	C. C.	20 months	,,	+	±	−	−	−	−
98	C. C.	?	,,	+	±	−	−	−	−
99	A. C.	3 months	,,	+	±	−	−	−	−
100	V. C.	4½ years	,,	+	±	−	−	−	−
101	R. C.	1½ ,,	,,	+	±	−	−	−	−
102	C. G.	4½ ,,	,,	+	±	−	−	−	−
103	A. M.	7 ,,	,,	+	±	−	−	−	−
104	E. R.	6 ,,	,,	+	±	−	−	−	−
105	V. T.	3 months	,,	+	±	−	−	−	−
106	D. A.	2 years	,,	+	+	−	−	−	−
107	C. C.	Native	,,	+	+	−	−	−	−
108	G. C.	,,	Oct. to Dec., 1905	+	+	−	−	−	−
109	G. N.	12 years	Negative	+	+	−	−	−	−
110	A. S.	?	,,	+	+	−	−	−	−
111	C. B.	5 months	,,	+	+	+	−	−	−
112	C. B.	Native	,,	+	+	+	−	−	−
113	G. C.	9 months	,,	+	+	+	−	−	−
114	M. C.	16 years	,,	+	+	+	−	−	−
115	F. D.	1½ ,,	,,	+	+	+	−	−	−
116	R. G.	9 months	,,	+	+	+	−	−	−

117	G. L.	1¼ years	,,	+	+	+	−	−	−
118	C. M.	Native	,,	+	+	+	−	−	−
119	R. M.	,,	,,	+	+	+	−	−	−
120	R. T.	5 years	*	+	+	+	−	−	−
121	G. T.	6 ,,	Negative	+	+	+	−	−	−
122	G. C.	4 ,,	,,	+	+	+	+	−	−
123	A. C.	Native	,,	+	+	+	+	−	−
124	F. C.	,,	,,	+	+	+	+	−	−
125	G. D.	5 years	,,	+	+	+	+	−	−
126	M. D.	3 ,,	,,	+	+	+	+	−	−
127	G. S.	6 ,,	,,	+	+	+	+	−	−
128	F. S.	1¼ ,,	,,	+	+	+	+	−	−
129	C. G.	Native	Sept. to Dec., 1904	+	+	+	+	+	−
130	A. M.	,,	Negative	+	+	+	+	+	−
131	A. M.	3½ years	,,	+	+	+	+	+	−
132	A. T.	2 months	,,	+	+	+	+	+	−
133	F. T.	10 years	,,	+	+	+	+	+	−
134	B. A.	5 months	Jan., 1906	+	+	+	+	+	+

* Rheumatism (?) March, 1906.

tri-monthly medical examination is carried out by certain district medical officers.

Preliminary enquiries elicited a definite history of an attack of Malta Fever varying in duration from two to twelve weeks in 3, and a history of "rheumatism" suspiciously like the neuritis associated with the Fever in 1, out of a total of 147 registered women. Next, specimens of blood were collected from 134 of those previously interviewed—the remaining 13 refusing to allow either finger or ear to be pricked in order to obtain samples—for the purpose of carrying out the agglutination test. Of these 134, 93 (or 69·4 per cent.) gave a completely negative reaction, 12 (or 8·95 per cent.) a slight reaction, indicating probably either a recent extremely mild or very remote attack of Malta Fever, and 29 (or 21·64 per cent.) yielding reactions that pointed to present or recent more severe infections.

Combining these two latter classes, it will be seen that no less than 41 out of a total of 134, or 30·59 per cent., exhibited evidence of *M. melitensis* infection.

The next step was to obtain vaginal swabbings and specimens of urine from such of the prostitutes as yielded a positive serum reaction and, by means of plate preparations, endeavour to determine the presence or absence of *M. melitensis* from these situations.

Of the 41 women yielding positive serum reactions, five, finding their occupation gone owing to the absence of the Fleet from Malta, returned to their homes in Sicily, and further specimens could not be obtained from them; four more absolutely refused to permit swabbings or specimens of urine to be taken; the remainder, 32 in number, afforded specimens for examination on one, two, or three several occasions.

In the collection of these specimens the vaginal swabbings were taken on ordinary sterile swabs such as are employed for collecting diphtheritic material (a piece of absorbent wool twisted round the end of a 15-cm. length of iron wire, enclosed in a test-tube, and the whole sterilised by dry heat), from just within the vaginal orifice or from some portion of the lower third of the vaginal wall. A Sims speculum was then passed into the vagina and the mouth of the os uteri exposed, and some of the cervical mucus collected on other sterile swabs. With regard to the urine samples, it was rarely possible to obtain catheter specimens, and, consequently, after the vaginal swabbing had been taken, a sterile, wide-mouthed bottle was handed to the woman and she was instructed to pass water directly into it. In this way, although asepsis was somewhat neglected, specimens of urine remarkably free from extraneous germs were usually obtained.

On arrival at the Laboratory, plate cultivations were prepared from each of the specimens as follows:—

Vaginal or Cervical Swabbing.—With a pair of sterile forceps the end of the swab wire was bent up at right angles so as to form an L-shaped rod, the infected cotton-wool occupying the short arm. Using the instrument now as a spreader, the swabbing was smeared over the surface of three nutrose agar plates in succession, and the plates numbered in the order of their inoculation.

Urine.—(*a*) One-tenth of a cubic centimetre of the urine sample was evenly distributed over the surface of each of four nutrose agar plates by means of a sterile L-shaped glass rod.

(*b*) Twenty cubic centimetres of the urine sample were transferred to a sterile centrifuge tube and thoroughly centrifugalised. Nearly all the supernatant fluid was pipetted off and the deposit shaken up with the remaining half cubic centimetre of urine, taken up in a sterile pipette and divided between two Petri dishes of nutrose agar. By means of a glass spreader, the urine deposit was distributed all over the surface of the first plate, and with the same instrument two further nutrose plates were inoculated in series. The remainder of the urine deposit, placed on the second plate, was distributed over it in like manner by the help of a second spreader, and two further plates inoculated in series with it.

After inoculation and labelling with distinctive number, etc., all the plates were transferred to the incubator at 37° C., and kept under observation up to the end of seven days. All suspicious colonies were tested by means of a serum of high titre, and those reacting were verified by tinctorial and cultural tests.

The details of the examinations are given in tabular form (p. 112), but the results may be briefly summarised as follows:—

(1) *Urine.*—*M. melitensis* recovered on five occasions from four patients.

(2) *Cervical Mucus.*—*M. melitensis* never recovered from this situation.

(3) *Vaginal Swabbings.*—*M. melitensis* recovered on two occasions from one patient convalescent from an attack of Malta Fever, and on one occasion from a patient who had suffered from a severe attack 18 months previously.

In connection with the isolation of *M. melitensis* from vaginal swabbings, it is interesting to note that the micrococcus was isolated from the the same situation, and also from the milk and the urine in the case of a married woman, Mrs. A., about a week after her discharge as convalescent from the Married Families (Military) Hospital at Valletta.

In view of the foregoing results an attempt was made to determine experimentally the possibility of infection taking place through the genital mucous membrane—a possibility which was rendered the more probable by reason of Shaw's successful infections through the apparently intact conjunctival mucous membrane (see these Reports, V, p. 10).

Table XXXV.—Examination of Urine, etc.

No. (*cf.* Table XXXIV).	Initials.	Urine.		Vaginal swabbings.		Cervical mucus.	
		No. of examinations.	*M. melitensis* recovered.	No. of examinations.	*M. melitensis* recovered.	No. of examinations.	*M. melitensis* recovered.
94	N. A.	3	0	3	0	2	0
95	C. A.	1	0	1	0	1	0
100	V. C.	3	0	3	0	2	0
101	R. C.	3	0	3	0	2	0
103	A. M.	3	0	3	0	2	0
104	E. R.	1	0	1	0	1	0
106	D. A.	2	0	2	0	1	0
107	C. C.	3	0	3	0	2	0
108	G. C.	1	0	1	0	1	0
109	G. N.	2	0	2	0	2	0
111	C. B.	1	0	1	0	1	0
112	C. B.	2	0	2	0	2	0
115	F. D.	2	0	2	0	1	0
116	R. G.	1	0	1	0	1	0
117	G. L.	2	0	2	0	2	0
118	C. M.	2	0	2	0	2	0
119	R. M.	3	0	3	0	3	0
120	R. T.	2	0	2	0	1	0
121	G. T.	2	0	2	0	1	0
122	G. C.	1	0	2	0	1	0
123	A. C.	2	0	2	0	2	0
124	T. C.	1	0	1	0	1	0
125	G. D.	1	1	1	0	1	0
126	M. D.	2	0	2	0	1	0
127	G. S.	3	0	2	0	1	0
128	F. S.	2	0	2	0	2	0
129	C. G.	1	1	1	1	1	0
130	A. M.	3	0	3	0	2	0
131	A. M.	2	0	2	0	2	0
133	F. T.	2	2	2	0	1	0
134	B. A.	2	1	2	2	2	0

A prolonged search was first made for an infected milch goat which should combine the two factors—readiness for impregnation and the excretion of urine containing *M. melitensis*—but without success; consequently the demonstration of the infection of the healthy male goat as a sequent to covering the suitable infected female had perforce to be abandoned. Monkeys were available but proved refractory owing to the vicious nature of the females, and pairing was unsuccessful. Finally, two healthy male monkeys were selected; first one and then the other was securely held on the operating table, the glans penis exposed and carefully examined with a hand lens for scratches and abrasions. As the mucous membrane in each case appeared to be intact, the following experiments were made:—

(1) A strip of cotton-wool was first dipped in a fresh specimen of urine from an ambulatory case of Malta Fever (under observation in H.M. Naval Dockyard), then wrapped around the glans penis of Monkey No. 203 and left in contact with the mucous membrane for a period of half a minute. The wool was then removed, the glans mopped dry with sterile cotton-wool and the monkey returned to his cubicle.

The specimen of urine was then plated out and the subsequent growth showed the presence of 500 colonies of *M. melitensis* per cubic centimetre.

On the 17th day the blood serum of Monkey No. 203 in dilution of 1 in 20 caused typical clumping of *M. melitensis*. The titre of the serum gradually rose until on the 28th day it had reached 1 in 150. The animal was then killed with chloroform vapour, and at the *post-mortem* examination the micrococcus was recovered in large numbers from right and left inguinal glands, and in smaller numbers from the right axillary gland and the spleen, but was absent from the blood; the *post-mortem* also revealed the fact that this monkey was the subject of miliary tuberculosis.

(2) After exposing the glans of Monkey No. 200 it was first dried with sterile cotton-wool, and then a small area of the surface was rubbed with a dry, hard pad of sterile cotton-wool to produce a "friction" excoriation. A piece of wool soaked in the same specimen of urine that was used for the first experiment was wrapped around the glans and left in contact for 30 seconds. It was then removed, the glans dried and the monkey returned to his cage.

On the 17th day an agglutination reaction in dilution of 1 in 10 was obtained and by the 28th day the serum value had risen to 1 in 200. The animal was killed on this day and at the *post-mortem* the *M. melitensis* was recovered from the blood—to the number of about 10,000 per cubic centimetre—and in large numbers from the spleen, the axillary, inguinal, and mesenteric glands.

Both these experiments, therefore, yielded positive results, infection taking place in the first case by absorption of the coccus through an

Table XXXVI.—Infection of Monkey *viâ* Mucous Membranes.

No. of monkey.	Sex.	Condition of exposed surface of mucous membrane of glans penis.	Infective material.	Serum reaction.		Duration of experiment in days.	*Post-mortem* findings.							
				Day of appearance.	Amount of dilution.		Value of serum.	*M. melitensis* recovered from—						
								10 c. mm. of blood.	Axillary glands. R.	L.	Inguinal glands. R.	L.	Mesenteric glands.	Spleen.
203	♂	Apparently intact.	Urine from ambulatory case of Malta Fever (1857), see p. 128	17	1 : 20	28	1 : 150	—	+	—	+	+	—	+
200	♂	Friction excoriation.	Ditto	17	1 : 10	28	1 : 200	1000	+	+	+	+	+	+

apparently uninjured and intact mucous membrane, and in the second case probably through the artificially produced lesion—in both the incubation period extended to 17 days, an interval between exposure to infection and the first appearance of symptoms which tallies very closely with those noted by Shaw in the experiments previously referred to.

VII. PROPHYLACTIC INOCULATION WITH *M. melitensis* VACCINE.

The results that had attended the use of *M. melitensis* vaccine during the winter of 1905–6 by one of the members of the Working Party in the treatment of cases of Malta Fever after return to England, encouraged the hope that its employment as a prophylactic vaccine might be a measure of practical utility, and arrangements were made to put it to the test.

As the records of the Royal Naval Hospital at Bighi and of the R.A.M.C. Detachment in Malta showed an abnormally high case-incidence amongst those men whose duty it was to nurse Malta Fever patients, it was decided to limit the vaccination for the summer of 1906 to this section of the community. The plan being explained to the men, a sufficient number of the Sick Bay Staff and Nursing orderlies expressed their desire to submit to the inoculations, to admit of the full strength being divided into two nearly equal batches both at the Military and the Naval Hospitals—one section at each institution being untouched or injected merely with sterile saline solution to serve as controls.

The Subjects—Naval.—Of the staff of the Naval Hospital at Bighi, 43 men were available for observation in this connection, of whom 23 were vaccinated (7 on one occasion only and 16 on two occasions) and 20 were reserved as controls. From the 23 vaccinated men, however, two must be deducted, for the danger that must always exist in carrying out a series of vaccinations within the endemic area was here encountered and two of the men were vaccinated—each with a dose of 200 millions of cocci—during the *incubation period* of the

Table XXXVII.—Incidence of Malta Fever on the Bighi Hospital Staff.

	1901.	1902.	1903.	1904.	1905.	1906, Jan.—April.
Members of Sick Berth Staff exposed to infection	31	51	65	75	80	43
Number contracting Malta Fever...........................	5	19	12	16	12	13

naturally acquired disease. This leaves 21 inoculated men and 20 controls who were under observation from April 27th to August 30th, 1906. During this period one of the controls contracted the disease, but no cases occurred amongst the vaccinated men.

The incidence of Malta Fever on the Sick Berth Staff during previous years is shown in Table XXXVII.

Military.—The strength of the Valletta detachment of the Royal Army Medical Corps (R.A.M.C.) at the commencement of the experiment comprised 84 men, of whom 12 had already suffered from an attack of Malta Fever, and four were then in hospital. This left a balance of 68 available for the purposes of observation. Of these 30 volunteered for inoculation, the remaining 38 served as controls. The fallacies involved in such a division have already been threshed out in connection with prophylactic inoculations of typhoid vaccine, and need not be further enlarged upon here. Suffice to say, that of the inoculated men 15 received one injection only, and 15 received two injections; and during the four months, from April 26 to August 30, 1906, that the men were under the observation of the operator, two cases occurred amongst the non-vaccinated controls, and none in the vaccinated men.

At the beginning of September, however, two cases occurred in the vaccinated men. The first, Lance-Corporal Johnson, assistant in the Commission Laboratory at Valletta, who had been injected twice with a dose of 400 millions of cocci on each occasion, and whose serum value had reached 1 in 50, suffered a typical attack, and the second, Private Boyd, who had been inoculated once only with a similar dose, and whose serum had a value of 1 in 30.

Owing to the distribution of the units of the detachment over the whole island for duty at the various military hospitals, and the frequent changes of station that take place, it is difficult to tabulate the incidence of Malta Fever in that portion of the detachment stationed at Valletta Hospital. Dealing, however, with the period January 1 to April 30, 1906, in an average strength of 81 (including 12 who had already suffered from the disease), five cases of Malta Fever occurred, whilst during the period May 1 to August 21, 1906, with a strength similar in numbers and composition, only two cases, both in non-vaccinated controls, occurred.

The Vaccine.—The vaccine employed was the remainder of a batch prepared and bulbed on March 16, 1906, in the Bacteriological Department of Guy's Hospital. The method of preparation varied in no respect from that usually adopted, and may be briefly described, first premising that all apparatus and solutions employed must be absolutely sterile, and every operation carried out under strictly aseptic conditions and with every possible precaution to avoid the entrance of extraneous micro-organisms.

A virulent culture of *M. melitensis* direct from the spleen of a guinea-pig, dead from *M. melitensis* septicæmia within 24 hours of intracerebral inoculation, is carefully emulsified in about 5 c.c. of sterile saline solution and transferred to the glass reservoir of an aseptic "laryngeal spray." By means of this little spraying apparatus the emulsion is evenly distributed over the surface of the "optimum" reaction (+8) agar contained in each of several culture bottles of either the Roux or the Kolle pattern. After insemination the culture bottles are incubated aerobically at 37° C. for from 24 to 36 hours. At the end of the incubation period the growth in the culture bottles is examined visually to determine its freedom from gross contamination; by means of stained preparations to determine its purity; and by the agglutination test with serum of high titre to determine its identity. The culture proving satisfactory, 5 c.c. of a 0·1-per-cent. saline solution are pipetted into each bottle, and by means of gentle agitation caused to take into suspension the upper layers of micrococci in the growth.

The turbid fluid is then pipetted off into a flask, which is labelled "A," and which receives in turn the surface washings from each of the culture bottles. A further 5 c.c. saline solution is introduced into the culture bottle, and the remainder of the growth is emulsified as evenly as possible with the help of a curved glass or platinum rod; this very turbid emulsion is transferred to a second flask labelled "B," containing a number of glass beads. When this process has been repeated with all the rest of the culture bottles and the emulsion added to that already in the flask with the beads, the flask with its contents is vigorously shaken at short intervals during the next half hour or so in order to disintegrate as far as possible any clumps of micrococci that may be present. The emulsion is then transferred to centrifugal tubes, placed in an electrical centrifuge, and run at a speed of some 2500 revolutions per minute for 30 minutes. At the end of this time a considerable deposit will have been thrown down, which is left behind in the tube, while the supernatant fluid is pipetted off and added to the surface washings from the culture bottles in the first flask. The centrifugal deposit is usually destroyed, but if a large quantity of vaccine is urgently required the deposit is returned to the flask "B," a few cubic centimetres of the saline solution added, and the shaking with the beads repeated. Further small quantities of saline solution are added from time to time during the process until an emulsion is formed which, to the naked eye, presents an opacity rather denser than that present in the emulsion in flask "A." This emulsion is again centrifugalised, and the supernatant fluid may be pipetted off and added to the emulsion in flask "A."

The emulsion in flask "A" is now ready for the enumeration of its contained micrococci, which is done by Wright's method if time is of supreme importance.

Wright's method consists of taking into a capillary Pasteur pipette, furnished with a rubber teat, three equal volumes of a solution containing 0·75-per-cent. sodium chloride and 1-per-cent. sodium citrate, one volume of normal blood direct from a needle puncture of the finger or ear (and assumed to contain 500 millions of red cells per cubic centimetre), and one similar volume of the emulsion of cocci. These various fluids are ejected from the pipette on to a slide and mixed thoroughly by repeatedly aspirating into and ejecting from the pipette; finally, the mixture is transferred to a clean slide, and with the help of a second slide a blood film is prepared in the usual way. This is stained by Jenner's or by Leishman's stain, and examined microscopically with a 1/12-inch objective; then by the enumeration of the red cells and of the micrococci respectively in a number of "fields" an average is struck for the ratio of the one to the other, and from this ratio is calculated the number of micrococci present per cubic centimetre of the emulsion.

Whenever possible it is desirable to control this method by ordinary plating methods—after such dilution of the emulsion as may be deemed necessary—incubating for three days at 37° C., and enumerating the resulting colonies by the help of a counting disc.

Whilst the enumeration is in progress the emulsion is distributed, in quantities of 10 c.c., in as many sterile test-tubes plugged with cotton-wool as may be necessary; the tubes are then suspended on a water bath running at 60° C., and allowed to remain there for half an hour from the time the water in the bath rose again to 60° C. after its temporary fall consequent upon the introduction of the tubes. At the end of this time the tubes are removed from the water bath and suspended in a bath of running water to cool them down rapidly. Next 0·1 c.c. of emulsion is removed from each of several tubes, and surface plate cultivations established from each sample in order to demonstrate the absence of living cocci from the emulsion. The tubes of crude vaccine are then protected by indiarubber caps slipped over the cotton-wool plugs, and set aside in the ice chest until the result of the enumeration is known.

Having measured the bulk of the crude vaccine and determined the number of cocci present per cubic centimetre, a very simple calculation decides the amount of diluent to be added to the emulsion to reduce the number of cocci present per cubic centimetre to, say, 1000 millions, which is, perhaps, the most useful standard. The diluent employed is a 0·1-per-cent. saline solution, the necessary quantity of which is measured out into a large flask, and to it is added tricresol in such amount that the finished vaccine shall contain 0·25 per cent. of the disinfectant, tricresol being employed in preference to phenol or lysol on account of its lack of irritating properties.

The crude vaccine is now transferred by means of sterile pipettes from the tubes in which the emulsion was sterilised to the flask containing the diluent and antiseptic, in which thorough mixture is effected. This flask is next connected up to the side feed of a graduated burette, and the vaccine filled into glass bulbs in quantities of 0·5 and 1 c.c. as required, the bulbs sealed off, in the blow-pipe flame, and labelled, with a writing diamond, with some distinguishing character or number, which indicates the character and number of cocci in suspension inside the bulb.

The Inoculations: Time.—The inoculation was usually performed the last thing at night (10 P.M.), so that several hours' rest in bed intervened before the heavy work of the following day commenced. In many instances, however, 11 A.M. or noon was the hour at which the injection was made, but so far as could be ascertained, the time at which the inoculations were carried out exercised no influence upon the immediate clinical results.

Site.—The site of inoculation was the subcutaneous tissue of the abdominal wall just below the costal margin. The skin in this situation was prepared by thoroughly cleansing a small area with a 2-per-cent. solution of lysol applied with cotton-wool, then removing all trace of the antiseptic by directing a stream of ether from a drop bottle on to the spot, or swabbing it well with a pad of wool saturated with ether. The ether served a subsidiary purpose, in that by its rapid evaporation it cooled the prepared area of skin and reduced its sensibility.

Dose.—The inoculations were made with the help of a hypodermic needle attached to a 10 or 20 c.c. syringe, holding 20 or 40 doses respectively, as the bulk of inoculum was invariably 0·5 c.c. By using a large syringe supplied with a number of spare hypodermic needles, it was only necessary to replace the soiled needle by a fresh sterile one, after each inoculation, and proceed to the next individual, thus saving much valuable time when many men had to be inoculated.

The vaccine brought from England for the purpose of these inoculations was numerically stronger than usual, and contained 2500 million cocci per cubic centimetre, so that the number of organisms per dose could readily be varied by suitable dilution of the emulsion.

The initial dose varied slightly in individual cases, but was either two, three, or four hundred millions of cocci. Subsequent doses were regulated by the response of the individual to inoculation, as judged by the movements of the curve representing the agglutinin-titre of the serum, but were usually 400 millions of cocci.

After the hypodermic injection of the vaccine, a wisp of cotton-wool and a drop of flexile collodion sealed the needle puncture and supplied all the dressing necessary.

In many of the cases two inoculations were given at suitable intervals.

Sequelæ of Inoculations.—Before giving the details of the inoculations carried out, and their results, a few words are necessary as to the local and constitutional effects following the introduction of the dead bodies of *M. melitensis* into the subcutaneous tissue of the normal human subject, as observed in these 51 men.

Local Appearances.—The introduction of such a small quantity of fluid into the subcutaneous tissue produced no immediate effect other than a slightly marked prominence of the skin at the seat of inoculation. Usually the emulsion was completely absorbed within a few hours, and on the morning following the inoculation no local alteration was discernable. In three of the orderlies of the R.A.M.C., the first inoculation was followed by a very distinct, hard, tender lump at the seat of inoculation, the skin over the swelling being red and slightly œdematous. Some tenderness of the inguinal and axillary glands was present so long as the lumps remained tender, but this passed off rapidly when the swelling had disappeared. In all three cases the swelling subsided without interference, the duration of a visible tumour being limited to two or three days respectively in the first two cases. In the third, the lump, which was on the right side, remained visible and tender for seven days, and was probably due to the fact that the subject—an ardent cricketer—was on bowling for practically the whole of the afternoon following inoculation. Several of the Sick Berth Staff of the Bighi Hospital complained of pain at the site of the needle puncture after the first injection, but in each case this was found to be due to the pull of the shrinking collodion on the surrounding fine hairs and not to any alteration in the tissues at the point where the emulsion of cocci was deposited. Inoculations subsequent to the first failed to cause any discomfort. The inguinal glands in many were slightly tender for the 24 hours following an injection, but not longer.

Constitutional Symptoms.—Beyond slight headache and feeling of malaise, associated with a rise of temperature to 98°·6 F. or 99° F., complained of by a few on the day following inoculation, no constitutional symptoms were observed. Two of the controls, however, who had been injected with normal saline solution complained of severe headache and showed temperatures of 99°·6 F. and 99°·8 F. respectively on the day following the injection. It will thus be seen that none of the disquieting results recorded by Lieut. Bousfield, R.A.M.C.,* were noted in this series of inoculations. That they do occur, however, was well shown in the case of one of the members of the Working Party who was inoculated by Lieut.-Colonel Leishman immediately prior to leaving England. Marked enlargement, accompanied by tenderness on pressure of the superficial lymphatic glands was observed within 24 hours of inoculation, together with some headache and malaise.

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* Bousfield, L, "Some Remarks on Protective Inoculation against Malta Fever," 'Journ. R.A.M.C.,' vol. 7, 1905, pp. 179—182.

Table XXXVIII.—Results of Inoculation with *M. melitensis* Vaccine, Royal Naval Hospital, Bighi.

No.	Number of times vaccinated.	Serum value before inoculation.	1st inoculation.		2nd inoculation.		Serum value (28/8/06) 4 months later.
			Dose of vaccine.	Agglutinin response.	Dose of vaccine.	Agglutinin response.	
1	1	nil	200	1 : 100 +	—	—	1 : 10 +
2	2	,,	300	1 : 10 +	400	1 : 10 +	1 : 50 +
3	2	,,	300	1 : 50 +	400	1 : 50 +	1 : 100 +
4	2	,,	300	1 : 100 +	400	1 : 100 +	1 : 50 +
5	1	1 : 10 ±	200	1 : 100 +	—	—	nil
6	1	nil	200	1 : 100 +	—	—	1 : 5 +
7	2	,,	200	1 : 50	400	1 : 100 +	1 : 10 +
8	2	,,	200	nil	400	1 : 10 +	nil
9	2	,,	200	1 : 100 +	400	1 : 200 +	1 : 10 ±
10	2	,,	300	1 : 10 +	400	1 : 10 +	1 : 10 +
11	1	1 : 10 ±	200	1 : 200 +	—	—	1 : 10 +
12	2	nil	300	1 : 50 ±	400	1 : 10	1 : 25 +
13	2	1 : 10 ±	200	nil	400	nil	nil
14	2	nil	300	1 : 50 ±	400	1 : 10 +	1 : 5 ±
15	2	,,	200	1 : 100 +	200	1 : 100 +	1 : 50 +
16	2	,,	200	nil	400	1 : 100 +	1 : 5 +
17	2	,,	300	1 : 50 +	400	1 : 50	1 : 25 +
18	2	,,	300	1 : 10 +	400	1 : 10 +	1 : 10 +
19	1	,,	300	1 : 100 ±	—	—	1 : 25 +
20	1	,,	250	1 : 10 +	—	—	1 : 10 +
21	1	,,	250	1 : 10 +	—	—	1 : 10 +

Table XXXIX.—Results of Inoculation with *M. melitensis* Vaccine, Royal Military Hospital, Valletta.

No.	Number of times vaccinated.	Serum value before inoculation.	1st inoculation.		2nd inoculation.		Serum value (28/8/06) 4 months later.
			Dose of vaccine.	Agglutinin response.	Dose of vaccine.	Agglutinin response.	
			millions.				
1	2	nil	200	nil	400	1 : 20 ±	nil
2	1	,,	400	1 : 100 +	—	—	—
3	2	,,	200	nil	400	1 : 10 +	1 : 10 ±
4	2	1 : 10 +	400	1 : 20 +	400	1 : 50 +	1 : 20 +
5	2	nil	400	1 : 20 +	400	1 : 20 +	1 : 10 +
6	1	,,	400	1 : 100 +	—	—	1 : 20 +
7	2	,,	200	nil	400	nil	nil
8	2	,,	200	nil	400	1 : 20 +	nil
9	2	1 : 10 +	200	1 : 20 +	400	1 : 20 +	1 : 50 +
10	1	nil	400	1 : 50 +	—	—	1 : 10 +
11	1	,,	400	1 : 50 +	—	—	1 : 10 +
12	1	,,	400	1 : 100 +	—	—	1 : 20 ±
13	2	,,	400	1 : 10 +	400	1 : 50 +	1 : 10 ±
14	1	,,	400	1 : 20 +	—	—	1 : 20 ±
15	2	,,	400	1 : 10 +	400	1 : 20 +	nil
16	1	,,	400	1 : 10 +	—	—	1 : 10 +
17	2	,,	400	1 : 20 ±	400	1 : 10 +	nil
18	1	,,	400	1 : 100 ±	—	—	nil
19	2	,,	400	1 : 10 +	400	1 : 100 ±	1 : 50 ±
20	2	,,	200	nil	400	1 : 20 +	nil
21	1	,,	400	1 : 50 +	—	—	1 : 20 +
22	1	,,	400	1 : 100 +	—	—	1 : 20 +
23	1	,,	400	1 : 50 +	—	—	1 : 20 ±
24	2	,,	200	1 : 10 ±	400	1 : 50 +	1 : 10 +
25	1	,,	400	1 : 10 +	—	—	1 : 10 +
26	2	,,	400	1 : 50 +	—	—	1 : 10 +
27	1	,,	400	1 : 20 ±	—	—	1 : 20 +
28	2	,,	200	1 : 20 +	400	1 : 50 ±	1 : 50 ±
29	1	,,	400	nil	—	—	nil
30	1	,,	400	1 : 20 +	—	—	1 : 20 ±

The seat of inoculation was occupied by a raised, hard, and tender lump, which gradually became soft and boggy to the touch, and the skin over it acquired a dusky-red colour. As there was every evidence of pointing, the tumour was incised on the 11th day and some thin serous pus, which proved sterile, was evacuated; and no higher dilution of the blood serum than 1 in 10 would give a positive agglutination reaction.

Later on an injection of the same brew of vaccine that was used in the series of prophylactic vaccinations now under consideration was introduced into the opposite flank. This was followed by a precisely similar train of events, although on this occasion the symptoms were distinctly less severe: the agglutination response on this occasion rose to 1 in 40.

Returning once more to the series of inoculations carried out upon men of the Naval Hospital and of the R.A.M.C. it must be noted that the response of the individual as judged by the movements of the agglutination value of the serum varied within wide limits from a positive reaction in a dilution of 1 in 200 to a negative reaction in a dilution of 1 in 5 as a result of the first injection.

A further point to be noted in the tabulated details is the very distinct fall in the titre of the serum that has taken place by the end of the four months, in one case from 1 in 200 to 1 in 10, showing that the individual response to vaccination, so far as relates to the production of antibodies of the agglutinin group at any rate, is limited to a comparatively short period, and apparently indicates that a further inoculation is necessary at the end of about three months after the first or second.

The immunity of the inoculated men during a period of four months from any attack of Malta Fever, though possibly due in part to the elimination of the "goat's-milk" factor, points to the necessity of a further trial of the *M. melitensis* vaccine—on a large scale; and in this case it would be of advantage to inoculate a large body of men, say half a regiment, before they enter the endemic area and then again after some three months' service in Malta.

VIII. CLINICAL OBSERVATIONS.

The limited time at the disposal of those members of the Working Party who were engaged in the Bacteriological and Experimental Investigation, no less than the paucity of cases occurring during the summer of 1906, precluded any extensive observations being carried out in connection with the clinical side of Malta Fever as it occurs in man. Certain points were, however, enquired into, and the results obtained are inserted here rather as indications of lines of research requiring further elucidation, than as dogmatic statements from which definite conclusions can be drawn.

Under these circumstances a bald outline of the observations made and the results obtained are all that are necessary, and these may be arranged under separate headings for convenience of reference.

(a) *Blood.—Presence of Micrococci therein.*—The presence and multiplication of *M. melitensis* in the blood of Malta Fever patients at various stages of the disease is now an established fact and fixes the position of the infection as a definite and specific septicæmia, though the researches of Gilmour and Shaw appear to indicate that the number of cocci present per cubic centimetre in the peripheral blood is usually small. A consideration of the work of these observers, however, led to the conclusion that if cases in the very early stages of the disease, at or near the summit of a period of pyrexia, were selected for observation and blood collected late in the day, relatively more organisms would be found than had hitherto been noted. A careful scrutiny of the case notifications enabled three cases to be eventually selected as especially suitable, and one observation was made on each patient.

The technique of the blood collection was that usually employed in hospital work in England; that is to say, the forearm was carefully washed, lathered with soft soap and shaved, then washed with lysol (2-per-cent. solution) and finally cleansed with ether, and a bandage was bound tightly about the arm above the bend of the elbow. The interior of a 5-c.c. all-glass syringe was next moistened with 10-per-cent. solution of sodium citrate in normal saline by first filling and then emptying the barrel; the object, of course, being to prevent any alteration in the physical condition of the collected blood, such as general or local clotting, between the moment of filling the syringe and of transferring the blood to the nutrient medium. The needle was then thrust into the lumen of either the median basilic or median cephalic vein and the syringe itself depressed and allowed to fill with blood as the piston was gradually withdrawn by gravity.

A series of test-tubes filled with accurately measured quantities of nutrient broth had previously been arranged in a test-tube rack. One cubic centimetre of the blood was then added to 9 c.c. of nutrient broth in the first test-tube and thoroughly mixed—this tube was labelled No. I. From Tube No. I, 0·1 c.c. of the mixed blood and broth was removed by a sterile graduated pipette and added to 9·9 c.c. broth in Tube No. II; 0·1 c.c. of the contents of Tube No. II were then added to 9·9 c.c. broth in Tube No. III. Next 1 c.c. of the contents of Tube No. I was added to a fresh tube of broth labelled Tube *1*: then 0·1 c.c. from Tube No. I was added to another tube of broth numbered Tube *2*. This process was then repeated with the two other tubes marked with Roman numerals, the Arabic figures *3*, *4*, *5*, and *6* being employed to distinguish the secondary tubes inoculated therefrom.

Thus a series of dilutions was obtained as under:—

		Cubic centimetres of blood.
Tube I	contained	1 (approximately)
" *1*	"	0·1
" *2*	"	0·01
" *3*	"	0·001
" *4*	"	0·0001
" *5*	"	0·00001
" *6*	"	0·000001

while Tubes Nos. II and III served as controls to Tubes *2* and *4* respectively.

Finally the 4 c.c. of blood remaining in the syringe were distributed amongst several tubes of broth to guard against the possibility of total failure to obtain a growth, which might otherwise happen if only a few cocci were present in the blood collected.

All the tubes were then incubated at 37° C. for preliminary enrichment, and on and after the third day plates were inseminated with broth from such of the tubes as showed naked eye growth.

Then by noting the tube containing the smallest quantity of blood from which the micro-organism was recovered it became an easy matter to calculate the number of cocci present per cubic centimetre in the peripheral blood of the patient.

By following this procedure the coccus was found to be present in all three cases in fairly large numbers. The following are the details of these cases :—

1. Private Kirton—onset of disease, 18.6.06 : admitted to hospital, 21.6.06. Blood collected, 25.6.06 at 7 P.M. Serum agglutination value—1 in 2000. *M. melitensis* present to the number of at least 1900 per cubic centimetre.
2. Private Fitzgibbon—onset of disease, 28.5.06 : admitted, 31.5.06. Blood collected, 5.7.06 at 7 P.M. Serum value—1 in 200. *M. melitensis* present to the number of 10,000 (but not 100,000) per cubic centimetre.
3. Private Kearney—onset of disease, 20.5.06 : admitted, 23.5.06. Blood collected, 5.7.06, at 7.30 P.M. Serum value—1 in 200. *M. melitensis* present to the number of 1000 (but not 10,000) per cubic centimetre.

2. *Leucocyte Formula.*—The work of the French observers in Tunis—Hayat, Cathoire, Cardaliguet, Schoull, etc.—tends to show that the leucocyte formula of the blood in Malta Fever cases presents a consistent variation from the normal, and to this variation is attached diagnostic importance. The variation is stated to be in the direction of a relative and often an absolute increase in non-granular cells; and the few

Table XL.—Blood Counts in Malta Fever.

Name.	Date of admission.	Type of case.	Date of blood examination.	Serum value.	Red cells per cubic millimetre.	Hæmoglobin.	Cell index.	White cells per cubic millimetre.	Differential count of white cells per centum.							
									Small lymphocytes.	Large lymphocytes.	Large hyaline mononuclear cells.	Polymorphonuclear neutrophiles.	Eosinophiles.	Mast cells.	Total non-granular cells.	Total granular cells.
Serg.-Maj. A.	30/6/06	Mild	27/7/06	1 : 500,000	4,870,000	p.c. 90	0·92	7600	47·6	3·6	3·4	45·0	0·0	0·4	54·6	45·4
Staff-Serg. B.	2/6/06	Severe	26/7/06	1 : 50	5,100,000	59	0·57	9000	19·8	2·4	5·0	70·0	2·2	0·6	27·2	72·8
Pte. K.........	2/6/06	Severe	26/7/06	1 : 2,000	4,670,000	68	0·72	7000	44·2	3·6	1·2	48·0	3·0	0·0	49·0	51·0
Rifleman J....	11/7/06	Mild	26/7/06	1 : 20	4,970,000	79	0·79	7000	42·8	3·6	2·0	50·2	0·6	0·8	48·4	51·6
	Normals		—	Nil	5,000,000	100	1·00	7500	24		5·0	70·0	0·5—1	?	29·0	71·0

observations made during the summer on this point afford some confirmation of these statements.

In four cases a complete blood examination was carried out, the red and white cells being enumerated by the aid of the Thoma Zeiss hæmatocytometer, the hæmoglobin estimated in a Gower's hæmoglobinometer, and a differential leucocyte count, made on films, stained by Jenner's method—the classification adopted under the last heading being that advocated by Price-Jones,* to whom we are greatly indebted for confirming the accuracy of the counts. The results obtained are tabulated in Table XL, from which it will be seen that in three there was a very marked rise in the non-granular cell-content of the blood. In the fourth the deviation from normal was only noticeable in the deficiency of hæmoglobin.

(b) *Milk.*—Although, arguing from analogy, it was a foregone conclusion that *M. melitensis* would be found in human milk, actual demonstration had hitherto been wanting on account of the infrequency of lactation being associated with Malta Fever. During the summer of 1906 opportunity was afforded of investigating three cases only, but from two of these the micrococcus was recovered.

In collecting specimens of the milk, the breast was first washed up with soap and water, then with ether, then the bell opening of a sterile glass breast pump was applied and the milk received in the side bulb. Details of the successful recoveries are as follows:—

Mrs. A., Military Family Hospital, Valletta. Onset of disease, 7.5.06. Date of delivery, 24.6.06. The flow of milk was scanty from the first and the mother was unable to suckle her infant, which was consequently hand-fed on condensed milk. A sample of milk was collected from the right breast 26.6.06. The milk, which was yellowish in colour and distinctly oily, gave a complete agglutination reaction in dilution of 1 in 100. Single drops (about 0·03 c.c.) were used to inseminate each of about a dozen plates, which after incubation yielded about half-a-dozen colonies of *M. melitensis* per plate, so that each cubic centimetre of milk contained about a couple of hundred micrococci. A second sample of milk collected 5.7.06, required the exercise of powerful suction before even a couple of cubic centimetres could be obtained. The agglutination reaction was now obtainable in dilution of 1 in 2000, and *M. melitensis* was present to the number of some 300 per cubic centimetre.

C. G., Misida. Attack of Malta Fever, September—December, 1904, during the puerperium. The milk practically dried up and the child was hand-fed. Eighteen months later (June, 1906) patient

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* Jones, C. Price, 'Brit. Med. Journ.,' 1905, vol. 1, pp. 409, 410; 1905, vol. 2, pp. 1112, 1113.

Table XLI.—*M. melitensis* in Urine of Ambulatory Cases.

Date of plating.	1857. B. Worley.		3414. F. Mallia.	
	Treatment.	Number of colonies of *M. melitensis* per c.c.	Treatment.	Number of colonies of *M. melitensis* per c.c.
April 20	nil	440	Daily from 27/4/06 to 1/6/06 peroxide of succinic acid 2 grs. p. aq. dist. ad. 3 x.	9,000
„ 24	„	1,800		2,000
„ 27	„	720		12,000
May 1	„	3,000		21,000
„ 4	„	185		300
„ 8	„	2,350		10,500
„ 11	„	1,800		12,000
„ 15	„	1,000		∞
„ 18	„	1,060		600
„ 22	„	860		600
„ 25	„	1,900		950
„ 29	„	∞		1,500
June 1	„	∞		∞
		on leave		
„ 12	„	40	nil	nil
„ 15	„	2,140	„	„
„ 19	„	180	„	„
„ 22	„	10	„	„
„ 26	„	150	„	„
„ 29	„	240	„	„
July 3	„	1,068	„	„
„ 6	„	3,890	„	„
„ 20	„	110	„	„
„ 24	„	2,555	„	„
„ 27	„	1,020	„	„
„ 31	„	2,210	„	„
Aug. 1—14	Daily from 18/8/06 to 31/8/06 peroxide of succinic acid, as Mallia	not enumerated, but *M. melitensis* constantly present	nil	nil
„ 21 ...		500	„	„
„ 24 ...		1,242	„	„
„ 28 ...		28	„	„
„ 31 ...		301	„	„
Sept. 4	nil	705	„	„
„ 7	„	6,300	„	„
„ 11	„	24,140	„	no sample
„ 14	„	14,000	„	nil
„ 18	„	3,500	„	„
„ 21	„	2,650	„	„
„ 25	„	6,715	„	„
„ 28	„	7,600	„	„

∞ = innumerable.

states that flow of milk has never entirely ceased—small quantities can still be squeezed from the gland. A sample of a couple of cubic centimetres collected in the sterile breast pump by the aid of a combination of suction and expression on three several occasions. The milk, which appeared normal in colour and consistence, gave a positive reaction in dilution of 1 in 200. Plate cultivations to the number of three were prepared, each with 0·5 c.c. of the milk at each examination. At the first and third *M. melitensis* could not be detected in the resulting growth, but at the second examination saprophytes were practically absent, and colonies of *M. melitensis* to the average number of 25 were present in each of the three plates, *i.e.*, average 50 per cubic centimetre.

(c) *Urine.*—The two cases of Ambulatory Malta Fever in workmen in R.N. Dockyard handed over by Staff-Surgeon Shaw, R.N., when he left Malta,* were kept under observation during the summer.

No. 3414, F. Mallia, was treated from 24.4.06 to 1.6.06 with peroxide of succinic acid in the form of a 1 in 10,000 solution, 5 ounces of the solution being administered morning and evening, and by June 15 the *M. melitensis* had disappeared from the urine.

No. 1857, B. Worley, who served as a control to the previous case whilst peroxide was being administered, continued to pass urine containing large numbers of *M. melitensis* until October, when the examination of his urine was discontinued, although he, too, had been put on the peroxide in similar doses for a fortnight from 18.8.06 to 31.8.06.

(d) *Fæces.*—Only one or two observations were made with a view to the detection of *M. melitensis* in the fæces. The results so far as concerned firm, formed motions, were negative, but in one fatal case (Private R.) of one and a-half months' duration, where the intestines were filled with light semi-fluid fæces, the presence of *M. melitensis* in considerable numbers was demonstrated after plating on ox serum nutrose agar.†

(e) *Post-mortem Examinations.*—But two fatal cases of Malta Fever occurred during the first eight months of 1906 in the R.M. Hospital, Valletta, upon which *post-mortem* examinations were held. The details of these are here tabulated.

Several *post mortems* (which were witnessed by the courtesy of the staff of the Civil Hospital) were conducted on fatal cases of Malta Fever, but in these systematic bacteriological investigations were not carried out.

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* See these Reports, IV, p. 12 and V, p. 40.

† Eyre, J. W. H., "The Preparation of Nutrose Agar," 'Trans. Path. Soc.,' vol. 55, 1904, pp. 91–105.

Table XLII.—*Post-mortem* Details.

Organ or tissue examined.	Pte. D. 27/1/06.	Pte. R. 31/5/06.
Heart blood	*M. melitensis* present	*M. melitensis* present
Pericardial fluid	not examined	,,
Spleen	*M. melitensis* present	,,
Bile	not examined	,,
Inguinal glands	,,	,,
Mediastinal glands	,,	absent
Mesenteric glands	*M. melitensis* present	*M. melitensis* present
Salivary glands	,,	not examined
Thyroid glands	,,	,,
Suprarenal glands	,,	,,
Pancreas	,,	,,
Fæces	not examined	*M. melitensis* present

IX. *Conclusions.*

It must be noted that, in addition to breaking new ground, much of the experimental work of the Mediterranean Fever Commission during the summer of 1906 has necessarily been arranged in order to elucidate the previous work of individual members and to collate the recorded facts; and from a general review of the whole question of *M. melitensis* infection in man, and in the light of our present knowledge, it may be fairly stated that—

(*a*) The most common method of infection is by the ingestion of infective articles of food—mainly milk.

(*b*) The next common path of infection is by subcutaneous inoculation during the handling of infective material—usually milk.

(*c*) More rarely infection may occur as the result of contagion, or, possibly, through convection by means of blood-sucking insects.

The results obtained by the Working Party during the summer of 1906 have been summarised in the foregoing pages in connection with the headings to which they relate; the more obvious and the more important of the conclusions arrived at are here recapitulated:—

1. The goat is highly susceptible to infection by *M. melitensis* as the result of cutaneous, subcutaneous, intravenous, and intraperitoneal inoculation, and as the result of feeding with infective material.

2. The course of the resulting septicæmia is extremely mild, and usually after the first few days cannot be demonstrated by clinical observation.

3. In order to detect *all* the infected milch goats in any given herd it is necessary to repeatedly examine the milk at short intervals. In selecting milks for bacteriological examination a positive milk agglutination reaction (Zammit's test) is a much more reliable indication

of the presence of *M. melitensis* than is a positive serum agglutination test.

4. During the course of the infection the *M. melitensis* first disappears from the peripheral blood and from most of the viscera, next from the spleen and kidneys, next from the superficial lymphatic glands, and last of all from the mammary gland.

5. The appearance of *M. melitensis* in the milk of the goat is a comparatively late phenomenon; the coccus present in this fluid is fully virulent, and the milk itself highly infective. The number of *M. melitensis* in the milk varies within wide limits from day to day, and bears no relationship to the severity of the infection, air temperature, etc.; the presence of *M. melitensis* in the milk appears to be merely the result of a mechanical flushing of the mammary gland by means of which the cocci multiplying therein are removed.

6. *M. melitensis* is not destroyed during the processes incident upon the manufacture of the ordinary ice-creams, or of the native cheeses, and may be present in the retail articles living and unaltered in virulence.

7. The association of infected milch goats in a herd with cases of Malta Fever in the owner's family (the members of which do not as a rule partake of the milk) suggests the frequency of cutaneous inoculation through scratches and abrasions of the human skin.

8. The administration by the mouth of infective goats' milk is usually followed in the monkey by an attack of *M. melitensis* septicæmia, 83 per cent. of the experimental animals yielding absolute and conclusive evidence *post mortem* of the existence of *M. melitensis* septicæmia, irrespective of the dose of infective bacteria.

9. The eight members of the crew of the "Joshua Nicholson," and one woman in the United States developed Malta Fever after the ingestion of infected milk under conditions which point to the absence of other sources of infection.

10. Weak solutions of hydrochloric acid exert some slight bactericidal power on *M. melitensis in vitro*, which becomes more marked when pepsin is also present in the solution. The introduction of infective milk directly into the stomach was not, in the two experiments made, followed by *M. melitensis* infection.

11. The mosquito (Acartomyia and Stegomyia) and the common blood-sucking fly (Stomoxys) can act as the host of *M. melitensis* for a short period, generally limited to four or five days, and during that time the bacterium retains its virulence unimpaired.

12. *M. melitensis* can exist for many days in, and be recovered in a virulent condition from, the "droppings" of these insects.

13. One only out of a number of experimental animals (in 14 of which exact data are available) showed signs *post mortem* of a mild

infection by *M. melitensis*, subsequently to being bitten by highly infective mosquitoes.

14. The existence of *M. melitensis* in urine and vaginal swabbings of infected females, together with the successful inoculation of experimental monkeys through the mucous membrane of the glans penis, points to the possibility of infection in man during sexual congress.

15. The inoculation of *M. melitensis* vaccine in the normal man is but rarely followed by any marked local or constitutional disturbance.

16. The prophylactic use of one or two doses of such vaccine in some 60 cases appears to have afforded protection for a period of about four months, and suggests the necessity of repeating this observation on a large scale—the first treatment to be carried out prior to the entrance of the men into the endemic area.

17. The therapeutic use of *M. melitensis* vaccine appears likely to become a valuable method in the treatment of the infected milch goats.

18. The few clinical observations that were made by the Bacteriological Section of the Working Party :—

(1) Demonstrated the presence of *M. melitensis* in human milk and fæces.

(2) Indicated the necessity for further investigation into the "leucocyte formula" in blood of Malta Fever cases in view of possible variations therein affording useful aid in diagnosis; and also for further investigation into the phenomenon of the passage of *M. melitensis* in the urine with a view to the selection of one or more urinary germicides capable of destroying the cocci in the genito-urinary tract.

RECOMMENDATIONS AS TO PREVENTIVE MEASURES IN CONNECTION WITH MEDITERRANEAN FEVER IN MALTA.

Various suggestions for the prosecution of a campaign against Mediterranean Fever arise from a consideration of the bacteriological and experimental data detailed in the Report of the work of the Mediterranean Fever Commission during 1906.

These fall naturally into one of two broad groups which, for our present convenience, may be designated by the titles of "palliation" and "eradication" respectively, and deal with the measures which must necessarily be adopted according to whether we merely wish to relieve our Navy and Army of the disastrous effects inseparable from the incidence of the disease, or to attempt the stamping out of Malta Fever from a dependency of the Crown which has, in the past, been considered of some strategic importance.

The first point to which attention should be directed, no matter what preventive measures are ultimately instituted, is the provision of an efficient and thorough system of compulsory notification of Mediterranean Fever. The system should probably be introduced in the form of an "Ordinance," and modelled on the lines of that at present in force in the British Isles under the Infectious Diseases (Notification) Act, 1889, and should be organised from a Central Office to which all notifications—naval and military as well as civil—should be sent. Provision should be made for the payment through the Public Health Department of fees (which in this instance need not exceed 6*d.* per notification) to civil practitioners in attendance on cases of Mediterranean Fever, and, on the other hand, for penalties for such as wilfully disregard the enactments of the Ordinance.

Palliation.—Reviewing the evidence already collected by the Mediterranean Fever Commission in its entirety, it is fairly obvious that the infective character of the milk of many of the goats upon the island of Malta affords a ready and reasonable explanation of the means by which the disease is transmitted. Then, too, the evidence yielded by experiments upon monkeys, supported by the facts of the s.s. "Joshua Nicholson" epidemic, justifies the assumption that in the ingestion of infected milk we have the veritable infective agency in the vast majority of cases. Additional weight attaches to this view by reason of the declining case incidence that was associated with the compulsory substitution (owing to the goatherds' strike) of imported preserved milks for the fresh goats' milk by the local naval and military authorities. Consequently, the strict prohibition of the use of the local supplies of fresh milk by the garrison and fleet should result in a very large diminution of the wastage from Malta Fever, and is a preliminary measure which must be enforced during the period that will necessarily elapse before the existing supply of virus is appreciably influenced by the measures to be suggested with a view to the eradication of the disease. Whether or no the time is yet ripe for the State supervision of the milk supplies, the erection of depôts, constructed upon sanitary principles, for the milking of goats and other animals, and the subsequent distribution of the milk, is an open question; but in any case the liberty of the goat to perambulate the streets of Valletta and other large towns, to feed from the garbage of the gutter, and to pollute the atmosphere, must be curtailed to the extent of prohibiting the entry of the animals into the fortified cities and barrack squares, if only to increase the effectiveness of regulations based upon the above recommendations.

Eradication.—The measures to be adopted under the preceding heading are comparatively simple, and when dealing with a disciplined body of men, such as compose our Navy and Army, fairly easily enforced. Measures for the stamping out of the disease, on the other hand, which have to be applied to the goat and other milk-yielding animals through the intermediary of the owner, must needs bring the sanitary authorities into conflict with the usually uneducated and often violently prejudiced native of the agricultural class, and it is uncertain how far recommendations under this head will, from the diplomatic point of view, commend themselves to the civil authorities. In the event of any or all such recommendations being adopted, the Ordinance upon which they are based will need to be administered with great firmness and tact.

In the first place, it is essential that all the goats in the island should be registered, and some method should be employed of numbering by means of stamped metal discs (such as is already carried out in the case of dogs), which will afford a ready means of identification of individual animals. Next, repeated analyses of samples of milk, taken at regular intervals, must be made in respect to every milch goat by medical officers of health (who need not be bacteriological experts) specially detailed for this purpose, at three, four, or more district "centres," where laboratories for the application of the Zammit milk test must be provided. Further, samples of the milk from each animal, giving a positive reaction with this test, must then be sent to the Central Laboratory in the Public Health Department at Valletta for bacteriological examination for the demonstration of the presence of *M. melitensis.* So far the suggested measures are simple in theory, and by no means costly in practice; nevertheless, unless carefully handled, the Maltese goatherd will offer considerable opposition to their execution.

The remaining measures deal with the segregation of such infected animals as are found from time to time to be yielding milk containing the specific micro-organism of Mediterranean Fever, and will necessarily be more expensive in operation, though, considering the importance of the issue at stake, not unduly so.

The total number of goats to be dealt with amounts approximately to 20,000, of which probably at least 2000 are infective, so that provision should be made on a liberal basis for dealing with possibly 5000 animals during the first year's work. When an animal is definitely shown to be yielding infective milk, it should be at once seized by the sanitary authorities and transferred to the Lazzaretto, or some other "pound" to be subsequently established, the owner being compensated on a scale comparable to that employed in Great Britain when infective animals (*e.g.*, in pleuro-pneumonia, tuberculosis, etc.) are seized by the public health authorities, and the milk stall should be disinfected and lime-washed. As, however, the flesh of infected goats, etc., is harmless when cooked, considerably more than half of the animals thus seized could be slaughtered, the viscera destroyed, and the flesh sold for consumption—a proceeding which would materially lighten the cost of these preventive measures. Moreover, as the progeny of infected milch goats is itself uninfected at birth, the seizure and destruction of infected animals should be combined in the case of the more valuable animals—the specially good milkers, etc.—with a modification of the "Bang process" for the reproduction of tuberculous herds, under the direct control of the Public Health Department, at special breeding farms where the selective in-breeding, which has rendered the Maltese milch goat such a valuable animal, could be continued under skilled supervision. This process, devised by Professor Bang, of the Copenhagen Veterinary College, as applied to tuberculous herds, has already been carried out with marked success in Denmark, Hungary, and the United States of America, and it appears probable that in dealing with the *M. melitensis* infection in goat the whole of the infective animals thus seized would be the replaced by healthy animals in the course of three or four years. Here, again, by the sale of yearlings a considerable return might be effected. While the capital sum required to organise and carry out all these measures in an efficient manner is greater than the civil authorities in Malta could readily devote to the purpose, the enormous saving in fighting material that would accrue to the Navy, and particularly to the Army, by the disappearance of Mediterranean Fever from the Maltese Islands, would amply justify an appeal to the Imperial Exchequer—assuming, of course, that Malta retains its present importance as a base for our sea and land forces.

Although, as is indicated later, certain powers are available already under Ordinance III of 1904, it would probably be preferable to introduce a new Ordinance for the purpose of stamping out Mediterranean Fever from the goats of the Maltese Islands, under the title of Infectious Diseases (Mediterranean Fever) Ordinance of 1907.

The various suggestions embodied in the foregoing paragraphs are here summarised.

REGULATIONS FOR THE PREVENTION OF MEDITERRANEAN FEVER.

I. *General.*

1. Compulsory notification enforced under an Ordinance which provides fees for compliance with, and penalties for disregard of, its provisions.

2. Popular education with regard to disinfection of excreta of patients suffering from Mediterranean Fever.

3. Prohibition, by Ordinance, of exportation (or importation) of goats, cows, ewes, asses, or other animals yielding milk for domestic purposes, which are the subjects of *M. melitensis* infection.

4. Prohibition of the entry of goats into Valletta and fortified cities, and into barrack squares, etc.

II. *Palliation.*

1. Popular education with regard to the infective character of the milk from certain animals with instructions as to boiling the fluid to destroy its noxious character; also with regard to the infective nature of milk products from such animals (*e.g.*, cheese, ice-cream).

2. Absolute prohibition, by orders from the Admiral commanding the Mediterranean Fleet and the officer commanding the land forces, of the purchase and consumption of native fresh milk (or of milk products, such as cheese, ice-cream, etc., prepared from native milk) by either officers or men, provision being made for definite penalties in the event of disobedience.

3. The official supply, by importation, of a sufficiency of reliable brands of preserved and condensed milks to the naval, military, and civil hospitals, and to the various canteens and messes.

III. *Eradication.*

1. Compulsory registration (by Ordinance) of every owner of one or more goats, cows, ewes, milch asses, etc., whether kept for the purpose of trade or otherwise, and of every breeder of such animals, and the granting of a metal badge bearing a specific sign or number for each animal, such badge to be securely attached to the animal to which it has been assigned, provision being made for the imposition of penalties for disregard or evasion of the enactments of this Ordinance.

2. Strict enforcement of Ordinance No. III of 1904, Cap. II, Section 5, Articles 79, 82, 83, and of the penalties referred to in Cap. IV, Articles 161 165, 174 (by Ordinance).

3. The interpolation of the words "milch goat, milch ass, ewe, or other animal yielding milk for domestic purposes" after the words "milch cow," in every article of the Fifth Section of Ordinance No. III of 1904, where such words are needed to apply the provisions of the article to animals other than the milch cow; also the interpolation of "Mediterranean Fever" into Article 91 (*b*), specifying certain infective diseases of animals; also the interpolation of the words "Mediterranean Fever" after the words "tuberculous disease" in Article 93, specifying powers of entry by sanitary officials, and of the words "or ice-creams" into Article 100 (2), specifying milk products to which the article is applicable.

4. The establishment of small laboratories with an adjoining compound, equipped for the performance of the "Zammit" milk test and the segregation of infected animals in such districts as Zeitun, Zurrik, Gargur, Notabile, and Mellieha in Malta and Victoria in Gozo, each under the charge of a specially detailed medical officer of health, assisted by a sanitary inspector; the function of each district laboratory being to examine once in every two weeks a sample of milk from each milch goat, etc., within its district; to seize, to segregate, and to report to the Public Health Department such animals as yield a positive reaction, and to forward specimens of their milk for bacteriological examination, and on the removal of infected animals to supervise the disinfection, etc., of the premises from which the seizure has been made.

5. On completion of proof that suspected animals are infected and infective, the Public Health Department shall seize such animals and dispose of them, either by slaughter and sale in the public abattoir, or otherwise, and compensate the owner according to a fixed scale to be subsequently determined.

6. In the case of valuable animals, good milkers and in good condition, the owner shall be compensated as in 5, and the animals retained alive for breeding purposes at an experimental station situated, say, at Ghain Tuffieha.

7. Immediately after the birth the offspring of infected dams shall be removed and subsequently fed on milk from healthy animals or on Pasteurised milk from infected animals.

8. As the infected animals are replaced by healthy ones they may be fattened up, slaughtered at the public abattoir, and the flesh sold.

9. Finally, experimental treatment by *M. melitensis* vaccine, on the lines indicated in this Report, should be carried out on a large scale, and its potentialities estimated.

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